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(54) DIFFERENTIATION-SUPPRESSIVE POLYPEPTIDE

(57) A polypeptide which contains the amino acid sequence described in SEQ ID NO: 1 in the Sequence Listing encoded by a gene originating in human being. Because of serving as a chemical efficacious in the supression of the proliferation and differentiation of undifferentiated blood cells, this polypeptide is expected to be usable in medicines and medical supplies.

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Description

Field of the invention

This invention relates to a novel bioactive substance which suppresses differentiation of undifferentiated cells.

Prior arts

Human blood and lymph contain various types of cells and each cell plays important roles. For example, the erythrocyte carries oxygen; platelets have hemostatic action; and lymphocytes prevent from infection. These various cells originate from hematopoietic stem cells in the bone marrow. Recently, it has been clarified that the hematopoietic stem cells are differentiated to various blood cells, osteoclasts and mast cells by stimulation of various cytokines in vivo and emvironmental factors. In the cytokines, there have been found, for example, erythropoietin (EPO) for differentiation to erythrocytes; granulocytecolony stimulating factor (G-CSF) for differentiation to leukocytes; and platelet growth factor (mpl ligand) for differentiation to megakaryocytes which is a platelet producing cells, and the former two have already been clinically applied.

The undifferentiated blood cells are generally classified into two groups consisting of blood precursor cells which are destined to differentiate to specific blood series and hematopoietic stem cells which have differentiation ability to all series and self-replication activity. The blood precursor cells can be identified by various colony assays, however identification method for the hematopoietic stem cells have not been established. In these cells, stem cell factor (SCF), interleukin-3 (IL-3), granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin-6 (IL-6), interleukin-1 (IL-I), granulocyte colony stimulating factor (G-CSF) and oncostatin M have been reported to stimulate cell differentiation and proliferation. Trials for expansion of hematopoietic stem cells in vitro have been examined in order to replace bone marrow transplantation for applying hematopoietic stem cell transplantation therapy or gene therapy. However, when the hematopoietic stem cells are cultured in the presence of the above mentioned cytokines, multi-differentiation activities and self-replication activities, which are originally in the position of the hematopoietic stem cells, gradually disappeared and are changed to the blood cell precursors which are only to differentiate to specific series after 5 weeks of cultivation, and multi-differentiation activity which is one of the specific features of the hematopoietic stem cells, is lost (Wagner et al. Blood 86, 512-523, 1995).

For proliferation of the blood precursor cells, single cytokine is not sufficient to effect, but synergistic action of several cytokines are important. Consequently, in order to proliferate the hematopoietic stem cells in maintaining with specific features of the hematopoietic stem cells, it is necessary to add cytokines which suppress differentiation together with the cytokines which proliferate and differentiate the undifferentiated blood cells. In general, many cytokines which stimulate proliferation or differentiation of cells are known, but small numbers of cytokines which suppressed cell differentiation are known. For example, leukemia inhibitory factor (LIF) has an action of proliferation of mouse embryonic stem cells without differentiation, but it has no action against the hematopoietic stem cells or blood precursor cells. Transforming growth factor (TGF - β) has suppressive action for proliferation against various cells, but no fixed actions against the hematopoietic stem cells or blood precursor cells.

Not only blood cells but also undifferentiated cells, especially stem cells are thought to be involved in tissue regeneration. These regeneration of tissues and poliferation of undifferentiated cells in each tissue can be applied in various ways by referring to the known reference (Katsutoshi Yoshizato, Regeneration- a mechanism of regeneration, 1996, Yodosha Publ. Co.).

Notch is a receptor type membrane protein which involves in regulation of nerve cells differentiation found in Drosophia. Homologues of the Notch are found in various animal kinds exceeding to the invertebrate and vertebrate including nematoda (Lin-12). Xenopus laevis (Xotch), mouse (Motch) or human (TAN-1). Ligand of the Notch in Drosophila are known. These are Drosophila Delta (Delta) and Drosophila Serrate (Serrate). Notch ligand homologues are found in various animal kinds as similar to the Notch of receptors (Artavanis-Tsakonas et al., Science 268, 225-232, 1995).

Human Notch homologue, TAN-1 is found widely in the tissues in vivo (Ellisen et al., Cell 66, 649-661, 1991). Two Notch analogous molecules other than TAN-1 are reported (Artavanis-Tsakonas et al., Science 268, 225-232, 1995). Expression of TAN-1 was also observed in CD34 positive cells in blood cells by PCR (Polymerase Chain Reaction) (Milner et al.. Blood 83, 2057-2062, 1994). However, in relation to humans, gene cloning of human Delta and human Serrate, which are thought to be the Notch ligand, have not been reported.

In Drosophila Notch, binding with the ligand was studied and invesgated in details, and it was found that the Notch can be bound to the ligand with CA^{++} at the binding region, which is a repeated amino acid sequence No. 11 and No. 12 in the amino acid sequence repeat of Epidemal Growth Factor (EGF) like repeating (Fehon et al., Cell 61, 523-534, 1990, Rebay et al., ibid. 67, 687-699, 1991 and Japan. Patent PCT Unexam. Publ. 7-503123). EGF-like repeated sequences are conserved in Notch homologues of the other species. Consequently, the same mechanism in binding with ligand is estimated. An amino acid sequence which is called as DSL (Delta-Serrate-Lag-2) near the amino acid ter-

minal, and EGF-like repeated sequence as like in the receptor are conserved in the ligand (Artavanis-Tsakonas et al., Science 268, 225-232, 1995).

The sequence of DSL domain is not found except for the Notch ligand molecules, and is specific to Notch ligand molecule. A common sequence of DSL domain is shown in the sequence listing, SEQ ID NO: 1 in general formula, and comparison with human Delta-1 and human Serrate-1 of the present invention and known Notch ligand molecules are shown in Fig. 1.

EGF-like sequence has been found in thrombomodulin (Jackman et al., Proc. Natl. Acad. Sci. USA 83, 8834-8838, 1986), low density lipoprotein (LDL) receptor (Russell et al., Cell 37, 577-585, 1984), and blood coagulating factor (Furie et al., Cell 53, 505-518, 1988), and is thought to play important roles in extracellular coagulation and adhesion.

Recently, the vertebrate homologues of the cloned Drosophila Delta were found in chicken (C-Delta-1) and Xenopus laevis (X-Delta-1), and it has reported that X-Delta-1 had acted through Xotch in the generation of the protoneuron (Henrique et al., Nature 375, 787-790, 1995 and Chitnis et al., ibid. 375, 761-766, 1995). Vertebrate homologue of Drosophila Serrate was found in rat as rat Jagged (Jagged)(Lindsell et al., Cell 80, 909-917, 1995). According to the Lindsell et al., mRNA of the rat Jagged is detected in the spinal cord of fetal rats. As a result of cocultivation of a myoblast cell line that is forced excess expressed rat Notch with a rat Jagged expression cell line, suppression of differentiation of the myoblast cell line is found. However, the rat Jagged has no action against the myoblast cell line without forced expression of the rat Notch.

Considering in the above reports, the Notch and ligand thereto may be involved in the differentiation regulation of the nerve cells, however, except some myoblast cells, their actions against cells including blood cells, especially primary cells, are unknown.

In the Notch ligand molecule, from the viewpoint of the prior studies on Drosophila and nematodae, the Notch ligand has specifically a structure of DSL domain which is not found other than in the Notch ligand. Consequently, the fact of having DSL domain means equivalent to ligand molecule for the Notch receptor.

Problems to be solved by the invention

As above mentioned, concerning with undifferentiated cells, proliferation for maintaining their specificities are not established. Major reasons are that factors suppressing differentiation of the undifferentiated cells are not found enough. Problems of the present invetion is to provide a compound originated from novel factors which can suppress differentiation of the undifferentiated cells.

Means for solving the problems

We have set up a hypothesis that the Notch and its ligand have action of differential regulation not only for neuroblasts and myoblasts but also for various undifferentiated cells, especially blood undifferentiated cells. However, in case of clinical application in the humans, prior known different species such as chicken or Xenopus laevos type notch ligand have problems species specificities and antigenicities. Consesquently, to obtain prior unknown human Notch ligand is essentially required. We had an idea that a molecule having DSL domain and EGF-like domain which are common to Notch ligand molecules and a ligand of the human Notch (TAN-1 etc.), which is a human Delta homologue (hereinafter designates as humnan Delta) and human Serrate homologue (hereinafter designates as humnan Serrate), may be found. Also we have an idea that these findings may be a candidate for drug useful for differential regulation of the undifferentiated cells. And we have tried to find out the same.

In order to find out human Notch ligands, we have analyzed amino acid sequences which are conserved in animals other than humans, and tried to find out genes by PCR using mixed primers of the corresponding DNA sequence. As a result of extensive studies, we have succeeded in isolation of cDNAs coding amino acid sequences of two new molecules, novel human Delta-I and novel human Serrate-1, and have prepared the expression systems of protein having various forms using these cDNAs. Also we have established purification method of the proteins which were purified and isolated.

Amino acid sequences of novel human Delta-1 are shown in the sequence listings, SEQ ID NO: 2-4. DNA sequence coding these sequence is shown in the sequence listing, SEQ ID NO: 8. Amino acid sequence of novel human Serrate-1 is shown in the sequence listings, SEQ ID NO: 5-7. DNA sequence coding these sequence is shown in the sequence listing, SEQ ID NO: 9.

Physiological actions of the these prepared proteins were searched by using nerve undifferentiated cells, preadipocytes, hepatocytes, myoblasts, skin undifferentiated cells, blood undifferentiated cells and immuno undifferentiated cells. As a result, we have found that novel human Delta-1 and novel human Serrate-1 had an action of differentiation-suppressive action to primary blood undifferentiated cells, and had a physiological action to maintain undifferentiated state.

Such actions to the blood undifferentiated cells have never been reported previously, and is a novel knowledge. No

significant toxic actions were noted in the toxicity studies on mice, and useful pharmaceutical effects were suggested. Consequently, the pharmaceutical preparations containing the molecule of the present invention, medium containing the molecule of the present invention, and the device immobilized with the molecule of the present invention are novel drugs and medical materials which can maintain the blood undifferentiated cells in the undifferentiated condition. Antibodies against human Delta-1 and human Serrate-1 are prepared by using antigens of the said human Delta-1 and human Serrate-1, and purification method of the said antibodies are established. The present invention has completed accordingly.

The present invention relates to a polypeptide comprising amino acid sequence of SEQ ID NO: 1 of the sequence listing encoded in a gene of the human origin, a polypeptide comprising at least amino acid sequence of SEQ ID NO: 2 or NO: 5 of the sequence listing, the polypeptide comprising amino acid sequence of SEQ ID NO: 3 of the sequence listing, the polypeptide comprising amino acid sequence of SEQ ID NO: 4 of the sequence listing, the polypeptide comprising amino acid sequence of SEQ ID NO: 6 of the sequence listing, the polypeptide comprising amino acid sequence of SEQ ID NO: 7 of the sequence listing, the polypeptide having differentiation suppressive action against undifferentiated cells, the polypeptide in which undifferentiated cells are the undifferentiated cells except for those of the brain and nervous system or muscular system cells, and the polypeptide in which undifferentiated cells are the undifferentiated blood cells. The present invention also relates to a pharmaceutical composition containing the polypeptides, and the pharmaceutical composition in which use thereof is hematopoietic activator. The present invention further relates to a cell culture medium containing the polypeptides, and the cell culture medium in which the cell is the undifferentiated blood cell. The present invention more further relates to a DNA coding a polypeptide at least having amino acid sequence of SEQ ID NO: 2 or NO: 5 of the sequence listing, the DNA having DNA sequence 242-841 of SEQ ID NO: 8 or DNA sequence 502-1095 of SEQ ID NO: 9 of the sequence listing, the DNA coding the polypeptide having amino acid sequence of SEQ ID NO: 3 of the sequence listing, the DNA having DNA sequence 242-1801 of SEQ ID NO: 8 of the sequence listing, the DNA coding the polypeptide having amino acid sequence of SEQ ID NO: 4 of the seqeuence listing, the DNA having DNA sequence 242-2347 of SEQ ID NO: 8 of the sequence listing, the DNA coding the polypeptide having amino acid sequence of SEQ ID NO: 6 of the seqeuence listing, the DNA having DNA sequence 502-3609 of SEQ ID NO: 9 of the sequence listing, the DNA coding the polypeptide having amino acid sequence of SEQ ID NO: 7 of the seqeuence listing, and the DNA having DNA sequence 502-4062 of SEQ ID NO: 9 of the sequence listing. The present invention still further relates to a recombinant DNA comprising ligating a DNA selected from the groups of DNA hereinabove and a vector DNA which can express in the host cell, a cell comprising transformed by the recombinant DNA, and a process for production of polypeptide by culturing cells and isolating the thus produced compound. The present invention still more further relates to an antibody specifically recognizing the polypeptide having the amino acid sequence of SEQ ID NO: 4 of the sequence listing, and an antibody specifically recognizing the polypeptide having the amino acid sequence of SEQ ID NO: 7 of the sequence listing.

The present invention is explained in details in the following.

Preparation of cDNA necessary for gene manipulation, expression analysis by Northern blotting, screening by hybridization, preparation of recombinant DNA, determination of DNA base sequence and preparation of cDNA library, all of which are series of molecular biological experiments, can be performed according to a description of the conventional textbook for the experiments. The above conventional textbook of the experiments is, for example, Maniatis et al. ed. Molecular Cloning, A laboratory manual, 1989, Eds., Sambrook, J., Fritsch, E.F. and Maniatis, T., Cold Spring Harbor Laboratory Press.

A polypeptide of the present invention has at least polypeptides in the sequence listing SEQ ID NO: 1 - 7. A mutant and allele which naturally occur in the nature are included in the polypeptide of the present invention unless the polypeptides of the sequence listing, SEQ ID NO: 1 - 7 lose their properties. Modification and substitution of amino acids are described in details in the patent application by the name of Benntt et al. (National Unexam. Publ. WO96/2645) and can be prepared according to the description thereof.

A DNA sequence coding polypeptides of the sequence listing, SEQ ID NO: 2 - 4 is shown in the sequence listing, SEQ ID NO: 8, and a DNA sequence coding polypeptides of the sequence listing, SEQ ID NO: 5 - 7 is show in the sequence listing, SEQ ID NO: 9, together with their amino acid sequences. In these DNA sequences, even if amino acid level mutation is not generated, naturally isolated chromosomal DNA or cDNA thereof may have a possibility to mutate in the DNA base sequence as a result of degeneracy of genetic code without changing amino acid sequence coded by the DNA. A 5'-untranslated region and 3'-untranslated region do not involve in amino acid sequence determination of the polypeptide, so DNA sequences of these regions are easily mutated. The base sequence obtained by these regeneracies of genetic codes is included in the DNA of the present invention.

Undifferentiated cells in the present invention are defined as cells which can growth by specific stimulation, and cells which can be differentiated to the cells having specific functions as a result of the specific stimulations. These include undifferentiated cells of the skin tissues, undifferentiated cells of the brain and nervous systems, undifferentiated cells of the muscular systems and undifferentiated cells of the blood cells. These cells include the cell of self-replication activity which is called as stem cells, and the cell having an ability to generate the cells of these lines. The

differentiation-suppressive action means suppressive action for autonomous or heteronomous differentiation of the undifferentiated cells, and is an action for maintaining undifferentiated condition. The brain and nervous undifferentiated cells can be defined as cells having ability to differentiate to the cells of the brain or nerve having specific functions by specific stimulation. The undifferentiated cells of the muscular systems can be defined as cells having ability to differentiate to the muscular cells having specific functions by specific stimulation. The blood undifferentiated cells in the present invention can be defined as cell groups consisting of the blood precursor cells which are differentiated to the specific blood series identified by blood colony assay, and hematopoietic stem cells having differentiation to every series and self-replication activities.

In the sequence listing, amino acid sequence in SEQ ID NO: 1 shows general formula of common amino acid sequence of DSL domain which is a common domain structure of the Notch ligand molecules, and at least this domain structure corresponds to the sequence listing, SEQ ID NO: 158 - 200 of the human Delta-1, or the sequence listing, SEQ ID NO: 156 - 198 of the human Serrate-1.

The amino acid sequence in the sequence listing, SEQ ID NO: 2 is a sequence of the active center of the present invention of human Delta-1 deleted the signal peptide, i. e. amino acid sequence from the amino terminal to DSL domain, and corresponds to an amino acid No. 1 to 200 in SEQ ID NO:4 of the matured full length amino acid sequence of human Delta-1 of the present invention. The amino acid sequence in SEQ ID NO: 3 is amino acid sequence of extracellular domain of the present invention of human Delta-1 deleted the signal peptide, and corresponds to an amino acid No. 1 to 520 in SEQ ID NO: 4 of the matured full length amino acid sequence of human Delta-1 of the present invention. The amino acid sequence of SEQ ID NO: 4 is the matured full length amino acid sequence of the human Delta-1 of the present invention.

The amino acid sequence in the sequence listing, SEQ ID NO: 5 is a sequence of the active center of the present inention of human Serrate-1 deleted the signal peptide, i.e. amino acid sequence from the amino terminal to DSL domain, and corresponds to an amino acid No. 1 to 198 in SEQ ID NO: 7 of the matured full length amino acid sequence of human Serrate-1 of the present invention. The amino acid sequence in SEQ ID NO: 6 is amino acid sequence of extracellular domain of the present invention of human Serrate-1 deleted the signal peptide, and corresponds to an amino acid No. 1 to 1036 in SEQ ID NO: 7 of the matured full length amino acid sequence of human Serrate-1 of the present invention. The amino acid sequence of SEQ ID NO: 7 is the matured full length amino acid sequence of the human Serrate-1 of the present invention.

The sequence of SEQ ID NO: 8 is total amino acid sequence of human Delta-1 of the present invention and cDNA coding the same, and the sequence of SEQ ID NO: 9 is total amino acid sequence of human Serrate-1 of the present invention and cDNA coding the same.

The left and right ends of the amino acid sequences in the sequence listings indicate amino terminal (hereinafter designates as N-terminal) and carboxyl terminal (hereinafter designates as C-terminal), respectively, and the left and right ends of the nucleotide sequences are 5'-terminal and 3'-terminal, respectively.

Cloning of human Notch ligand gene can be performed by the following method. During the evolution of the organisms, a part of amino acids sequences of the human Notch ligand is conserved. DNA sequence corresponding to the conserved amino acid sequence is designed, and is used as a primer of RT-PCR (Reverse Transcription Polymerase Chain Reaction), then a PCR template of the human origin is amplituded by PCR reaction, thereby fragments of human Notch ligand can be obtainable. Furthermore. RT-PCR primer is prepared by applying the known DNA sequence information of the Notch ligand homologue of the organisms other than humans, and the known gene fragments can be possibly obtained from PCR template of the said organisms.

In order to perform PCR for obtaining fragments of human Notch ligand, PCR for DSL sequence is considered, but a large number of combinations of DNA sequence corresponding to amino acid sequence conserved in this region can be expected, and a design for PCR is difficult. As a result. PCR of the EGF-like sequence has to be selected. As explained hereinbefore, since EGF-like sequence is conserved in a large number of molecules, to obtain the fragments and identification are extremely difficult.

We have designed and prepared about 50 PCR primer sets, for example the primer set of the sequence shown in Example 1, PCR was performed with these primer sets by using PCR template of cDNA prepared from poly A^+ RNA of various tissues of human origin, and more than 10 PCR products from each tissue were subcloned, as well as performing sequencing for more than 500 types. A clone having a desired sequence could be identified. Namely, the obtained PCR product is cloned in the cloning vector, transforming the host cells by using recombinant plasmid which contains the PCR product, culturing the host cells containing the recombinant plasmid in a large scale, purifying and isolating the recombinant plasmid, checking the DNA sequence of PCR product which is inserted into the cloning vector, and trying to obtain the gene fragment which may have a sequence of human Delta-1 by comparing with the sequence of the known Delta of other species. We have succeeded to find out the gene fragment which contains a part of cDNA of human Delta-1, the same sequence of DNA sequence from 1012 to 1375 described in the sequence listing, SEQ ID NO: 8.

We have also designed and prepared about 50 PCR primer sets, for example the primer set of the sequence shown

in Example 3, and PCR was performed with these primer sets by using PCR template of cDNA prepared from poly A^+ RNA of various tissues of human origin, and more than 10 PCR products from each tissue were subcloned, as well as performing sequencing for more than 500 types. A clone having a desired sequence could be identified. Namely, the obtained PCR product is cloned in the cloning vector, transforming the host cells by using recombinant plasmid which contains the PCR product, culturing the host cells containing the recombinant plasmid in a large scale, purifying and isolating the recombinant plasmid, checking the DNA sequence of PCR product which is inserted into the cloning vector, and trying to obtain the gene fragment which may have a sequence of human Serrate-1 by comparing with the sequence of the known Serrate of other species. We have succeeded to find out the gene fragment which contains a part of cDNA of human Serrate-1, the same sequence of DNA sequence from 1272 to 1737 described in the sequence listing. SEQ ID NO: 9.

A full length of the objective gene can be obtained from the human genomic gene library or cDNA library by using the thus obtained human Delta-1 fragment or human Serrate-1 gene fragment. The full length cloning can be made by isotope labelling and non-isotope labelling with the partial cloning gene, and screening the library by hybridization or other method. Isotope labelling can be performed by, for example, terminal labelling by using [^{32}P] γ-ATP and T4 polynucleotide kinase, or other labelling methods such as nick translation or primer extension method can be applied. In other method, human originated cDNA library is ligated into the expression vector, expressing by COS-7 or other cells, and screening the objective gene by expression cloning to isolate cDNA of the ligand. In the expression cloning, a cell sorter fractionation method which is applied with binding with polypeptide containing amino acid sequence of prior known 4 Notches such as TAN-1, and a detection method by film emulsion using radioisotope can be mentioned. In this specification, methods for obtaining genes of human Delta-1 and human Serrate-1 are explained, and in addition to that obtaining the Notch ligand homologue gene of the other organism is important for analysis of ligand action. This may be made by the same treatment. The obtained gene is subjected to DNA sequence determination and amino acid sequence can be estimated.

As shown in Example 2, gene fragments containing human Delta-1 PCR product is labelled with radioisotope to prepare hybridization probe, screened using cDNA of human placenta origin as the screening library, determined DNA sequences of the thus obtained clones, and obtained the clone containing DNA nucleotide sequence shown in the sequence listing, SEQ ID NO: 8, and shown it amino acid sequence coded in the sequence listing, SRQ ID NO: 4. We have succeeded cloning of cDNA coding full length of amino acids sequence of human Delta-1.

These sequences were compared with the data base (Genbank release 89, June, 1995), and found that these were novel sequence. The said amino acid sequence was analyzed in hydrophilic part and hydrophobic part according to a method by Kyte-Doolittle (J. Mol. Biol. 157: 105, 1982). A result indicated that human Delta-1 of the present invention is expressed on cells as a cellular membrane protein having a transmembrane domain.

As shown in Example 4, gene fragments containing human Serrate-1 PCR product is labelled with radioisotope to prepare hybridization probe, screened using cDNA of human placenta origin as the screening library, determined DNA sequences of the thus obtained clones, and obtained the clone containing DNA nucleotide sequence shown in the sequence listing, SEQ ID NO: 9, and shown it amino acid sequence coded in the sequence listing, SEQ ID NO: 7. In this screening, an intracellular part of gene sequence coding a full length of amino acids sequence, namely a peripheral part of termination codon can not be cloned. Consequently, as shown in Example 4, gene cloning is performed by RACE method (rapid amplification of cDNA ends, Frohman et al., Proc. Nati. Acad. Sci. U.S.A. 85, 8998-9002, 1988) and finally to succeeded cloning of cDNA coding full length of amino acid sequence of human Serrate-1.

These sequences were compared with the data base (Genbank release 89, June, 1995), and found that these were novel sequence. The said amino acid sequence was analyzed in hydrophilic part and hydrophobic part according to a method by Kyte-Doolittle (J. Mol. Biol. 157: 105, 1982). A result indicated that human Serrate-1 of the present invention is expressed on cells as a cellular membrane protein having a transmembrane domain.

Examples of plasmids integrated with cDNA are, for example. E. coli originated pBR322, pUC18, pUC19, pUC118 and pUC119 (Takara Shuzo Co., Japan), but the other plasmids can be used if they can replicate and proliferate in the host cells. Examples of phage vectors integrated with cDNA are, for example, λgt10 and λgt11, but the other vectors can be used if they can growth in the host cells. The thus obtained plasmids are transduced into suitable host cells such as genus Escherichia and genus Bacillus using calcium chloride method. Examples of the above genus E scherichia are Eseherichia coli K12HB101, MC1061, LE392 and JM109. Example of the above genus Bacillus is Bacillus subtilis MI114. Phage vector can be introduced into the proliferated E. coli by the in vitro packaging method (Proc. Natl. Acad. Sci., 71: 2442. 1978).

According to the analysis of amino acid sequence of the human Delta-1, amino acid sequence of a precursor of human Delta-1 consists of 723 amino acids residue shown in the sequence listing, SEQ ID NO: 8, and the signal peptide domain is estimated to correspond amino acid sequence of 21 animo acids residue from No.-21 methionine to No.-1 serine of the sequence listing; extracellular domain: 520 amino acids residue from No. 1 serine to No. 520 glycine; transmembrane domain: 32 amino acids residue from No. 521 proline to No. 552 leucine; and intracellular domain: 150 amino acids region from No. 553 glutamine to No. 702 valine. These domains are estimated domain construction from

amino acid sequences, and actual presence form may have possible to differ from the above structure, and constitutional amino acids of each domain hereinabove defined may have possibility to change 5 to 10 amino acids sequence.

According to a comparison in amino acid sequence of human Delta-1 and Delta homologue of the other organisms, the homologies with Drosophila Delta, chicken Delta and Xenopus laevis are 47.6%, 83.3% and 76.2%, respectively. The human Delta-1 of the present invention is different from these Deltas and is novel substance which is clarified at first by the present inventors. Search from all of organisms in the above data base indicated that polypeptides having the identical sequence of the human Delta-1 could not find out.

The homologues of Notch ligand have evolutionally conserved common sequence, i.e. repeated DSL sequence and EGF-like sequence. As a result of comparison with amino acid sequence of human Delta-1, these conserved sequence is estimated. Namely, DSL sequence conrresponds to 43 amino acids residue from No. 158 cysteine to No. 200 cysteine of the amino acid sequence in the sequence listing, SEQ ID NO: 4. EGF-like sequence exists with 8 repeats wherein, in the amino acid sequence in the sequence listing, SEQ ID NO: 4, the first EGF-like sequence from No. 205 cysteine to No. 233 cysteine; the second EGF-like sequence from No. 236 cysteine to No. 264 cysteine; the third EGF-like sequence from No. 271 cysteine to No. 304 cysteine; the fourth EGF-like sequence from No. 311 cysteine to No. 342 cysteine; the fifth EGF-like sequence from No. 349 cysteine to No. 381 cysteine; the sixth EGF-like sequence from No. 388 cysteine to No. 419 cysteine; the seventh EGF-1ike sequence from No. 426 cysteine to No. 457 cysteine; and the eighth EGF-like sequence from No. 464 cysteine to No. 495 cysteine.

A part of sugar chain attached is estimated from amino acid sequence of the human Delta-1 may be No. 456 asparagine residue in the sequence listing, SEQ ID NO: 4 as a possible binding site of N-glycoside bonding for N-acetyl-D-glucosamine. 0-glycoside bond of N-acetyl-D-galactosamine is estimated to be a serine or threonine residue rich part. Protein bound with sugar chain is generally thought to be stable in vivo and to have strong physiological activity. Consequently, in the amino acid sequence of polypeptide having sequence of the sequence listing, SEQ ID NO: 2, 3 or 4, polypeptides having N-glucoside or 0-glucoside bond with sugar chain of N-acetyl-D-glucosamine or N-acetyl-D-galactosamine is included in the present invention.

According to the analysis of amino acid sequence of the human Serrate-1, amino acid sequence of a precursor of human Serrate-I consists of 1218 amino acids residue shown in the sequence listing, SEQ ID NO: 9, and the signal peptide domain is estimated to correspond 31 amino acids residue in the amino acid sequence from No. -31 methionine to No. -1 alanine of the sequence listing; extracellular domain: 1036 amino acids residue from No. 1 serine to No. 1036 asparagine; transmembrane domain: 26 amino acids residue from No. 1037 phenylalanine to No. 1062 leucine; and intracellular domain: 106 amino acids domain from No. 1063 arginine to No. 1187 valine. These domains are estimated domain construction from amino acid sequences, and actual presence form may have possible to differ from the above structure, and constitutional amino acids of each domain hereinabove defined may have possibility to change 5 to 10 amino acids sequence.

According to a comparison in amino acid sequence of human Serrate-1 and Serrate homologue of the other organisms, the homologies with Drosophila Serrate, and rat Jagged are 32.1% and 95.3%, respectively. The human Serrate-I of the present invention is different from these Serrates and is novel substance which is clarified at first by the present inventors. Search from all of organisms in the above data base indicated that polypeptides having the identical sequence of the human Serrate-1 could not find out.

The homologues of Notch ligand have evolutionally conserved common sequence, i.e. repeated DSL sequence and EGF-like sequence. As a result of comparison with amino acid sequence of human Serrate-1 and other Notch ligand homologues, these conserved sequence is estimated. Namely, DSL sequence corresponds to 43 amino acids residue from No. 156 cysteine to No. 198 cysteine of the amino acid sequence in the sequence listing, SEQ ID NO: 7. EGF-like sequence exists with 16 repeats wherein, in the amino acid sequence in the sequence listing, SEQ ID NO:7, the first EGF-like sequence from No. 205 cysteine to No. 231 cysteine; the second EGF-like sequence from No. 234 cysteine to No. 262 cysteine; the third EGF-like sequence from No. 269 cysteine to No. 302 cysteine; the fourth EGF-like sequence from No. 309 cysteine to No. 340 cysteine; the fifth EGF-like sequence from No. 346 cysteine to No. 378 cysteine; the sixth EGF-like sequence from No. 385 cysteine to No. 416 cysteine; the seventh EGF-like sequence from No. 423 cysteine to No. 453 cysteine; the eighth EGF-like sequence from No. 462 cysteine to No. 453 cysteine; the nineth EGF-like sequence from No. 498 cysteine to No. 529 cysteine; the 10th EGF-like sequence from No. 536 cysteine to No. 595 cysteine; the 11th EGF-like sequence from No. 602 cysteine to No. 633 cysteine; the 12th EGF-like sequence from No. 640 cysteine to No. 671 cysteine; the 13th EGF-like sequence from No. 678 cysteine to No. 709 cysteine; the 14th EGF-like Sequence from No. 717 cysteine to No. 748 cysteine; the 15th EGF-like sequence from No. 755 cysteine to No. 786 cysteine; and the 16th EGF-like sequence from No. 793 cysteine to No. 824 cysteine. However, the 10th EGF-like sequence has irregular sequence containing 10 residues of cysteine.

A part of sugar chain attached is estimated from amino acid Sequence of the human Serrate-1 may be No. 112, 131, 186, 351, 528, 554, 714, 1014 and 1033 asparagine residue in the sequence listing, SEQ ID NO: 7 as a possible binding site of N-glycoside bonding for N-acetyl-D-glycosamine, 0-glycoside bond of N-acetyl-D-galactosamine is estimated to be a serine or threonine residue rich part. Protein bound with sugar chain is generally thought to be stable in

vivo and to have strong physiological activity. Consequently, in the amino acid sequence of polypeptide having sequence of the sequence listing, SEQ ID NO: 5, 6 or 7, polypeptides having N-glucoside or 0-glucoside bond with sugar chain of N-acetyl-D-glucosamine or N-acetyJ-D-galactosamine is included in the present invention.

As a result of studies on binding of Drosophila Notch and its ligand, amino acid region necessary for binding with ligand of Drosophila Notch with the Notch is from N-terminal to DSL sequence of the matured protein, in which signal peptide is removed (Japan. Pat. PCT Unexam. Publ. No. 7-503121). This fact indicates that a domain necessary for expression of ligand action of human Notch ligand molecule is at least the DSL domain, i.e. a domain containing amino acid sequence of the sequence listing, SEQ ID NO: 1, and a domain at least necessary for expression of ligand action of human Delta-1 is novel amino acid sequence shown in the sequence listing, SEQ ID NO: 2, and further a domain at least necessary for expression of ligand action of human Serrate-1 is novel amino acid sequence shown in the sequence listing, SEQ ID NO: 5.

A mRNA of human Delta-1 can be detected by using DNA coding a part or all of gene sequence in the sequence listing, SEQ ID NO: 8, and a mRNA of human Serrate-1 can be detected by using DNA coding a part or all of gene sequence in the sequence listing, SEQ ID NO: 9. For example, a method for detection of expression of these genes can be achieved by applying with hybridization or PCR by using complementary nucleic acids of above 12mer or above 16mer, preferably above 18mer having nucleic acid sequence of a part of sequence in the sequence listing, SEQ ID NO: 8 or 9, i.e. antisense DNA or antisense RNA, its methylated, methylphosphated, deaminated, or thiophosphated derivatives. By the same method, detection of homologues of the gene of other organisms such as mice or gene cloning can be achieved. Further cloning of genes in the genome including humans can be made. Using these genes cloned by such like methods, further detailed functions of the human Delta-1 or human Serrate-1 of the present invention can be clarified. For example, using the modern gene manipuration techniques, every methods including transgenic mouse, gene targeting mouse or double knockout mouse in which genes relating to the gene of the present invention are inactivated, can be applied. If abnomalities in the genome of the present gene is found, application to gene diagnosis and gene therapy can be made.

A transformant in which vector pUCDL-1F, which contains cDNA coding total animo acid sequence of human Delta-1 of the present invention, is transformed into E.coli JM109, has been deposited in the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, MITI, of 1-1-3, Higasi, Tsukuba-shi, Ibaragi-ken, Japan, as E. coli : JM109-pUCDL-1F. Date of deposit was October 28, 1996, and deposition No. is FERM BP-5728. A transformant in which vector pUCSR-1, which contains cDNA coding total animo acid sequence of human Serrate -1 of the present invention, is transformed into E.coli JM109, has been deposited in the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, MITI, of 1-1-3 Higasi, Tsukuba-shi, Ibaragi-ken, Japan, as E. coli : JM109-pUCSR-1. Date of deposit was October 28, 1996, and deposition No. is FERM BP-5726.

Exprssion and purification of various forms of human Delta-1 and human Serrate-1 using cDNA coding amino acid sequence of human Delta-1 and human Serrate-1 isolated by the above methods are known in the references (Kriegler, Gene Transfer and Expression- A Laboratory Manual Stockton Press, 1990 and Yokota et al. Biomanual Series 4, Gene transfer and expression and analysis, Yodosha Co., 1994). A cDNA coding the amino acid sequence of the isolated said human Delta-1 and human Serrate-1 is ligated to preferable expression vector and is produced in the host cells of eukaryotic cells such as animal cells and insect cells or prokaryotic cells such as bacteria.

In the expression of human Delta-1 and human Serrate-1 of the present invention. DNA coding polypeptide of the present invention may have the translation initiation codon in 5'-terminal and translation termination codon in 3'-terminal. These translation initiation codon and translation termination codon can be added by using preferable synthetic DNA adapter. Further for expression of the said DNA, promoter is linkaged in the upstream of the DNA sequence. Examples of vector are plasmid originated from Bacillus, plasmid originated from yeast or bacteriophage such as λ-phage and animal virus such as retrovirus and vaccinia virus.

Examples of promoters used in the present invention are any promoters preferable for corresponding to the host cells used in gene expression.

In case that the host cell in the transformation is genus Eseherichia, tac-promoter, trp-promoter and lac-promoter are preferable, and in case of host of genus Bacillus, SPO1 promoter and SP02 promoter are preferable, and in case of host of yeast, PGK promoter, GAP promoter and ADH promoter are preferable.

In case that the host cell is animal cells, a promoter originated from SV40 such as SRα promoter as described in Example, promoter of retrovirus, metallothionein promoter and heatshock promoter can be applied.

Polypeptide of the present invention can be expressed by using the expression vector having ability to be used by any person skilled in the arts.

Expression of the polypeptide of the present invention can be made by using only DNA coding the amino acid sequence of the sequence listing, SEQ ID NO: 2, 3, 4, 5, 6 or 7. However, the protein added with specific function can be produced by using DNA, to which added cDNA coding the known antigen epitope for easier detection of the produced polypeptide or added cDNA coding the immunoglobulin Fc for forming multimer.

As shown in Example 5, we have prepared expression vectors, which express extracellular proteins of human

Delta-1, as follow.

1) DNA coding the amino acids from No. 1 to 520 in amino acid sequence in the sequence listing, SEQ ID NO: 3,
2) DNA coding chimera protein to which added polypeptide having 8 amino acid, i.e. an amino acid sequence consisting of Asp Tyr Lys Asp Asp Asp Asp Lys (hereinafter designates FLAG sequence, the sequence listing, SEQ ID NO: 10), in the C-terminal of the amino acids from No. 1 to 520 in amino acid sequence in the sequence listing, SEQ ID NO: 3, and
3) DNA coding chimera protein to which added Fc sequence below the hinge region of human IgG1 (refer to International Patent Unexam. Publ. WO94/02053) in the C-terminal of the amino acids from No. 1 to 520 in amino acid sequence in the sequence listing. SEQ ID No:3, and to have dimer structure by disulfide bond in the hinge region,

are ligated individually with the expression vector pMKITNeo (Maruyama et al. Japan Molecular Biology Soc. Meeting Preliminary lecture record, obtainable from Dr. Maruyama in Tokyo Medical and Dental College, containing promoter SRα) to prepare extracellular expression vectors of human Delta-1.

The full-length expression vectors of the human Delta-1 as the expression vectors, which express full-length proteins of the human Delta-1, can be prepared as follows.

4) DNA coding amino acids from No. 1 to 702 in the sequence listing, SEQ ID NO: 4 and
5) DNA coding chimera protein to which added polypeptide having FLAG sequence in the C-terminal of amino acids from No. 1 to 702 in the sequence listing, SEQ ID NO: 4

are ligated individually with the expression vector pMKITNeo to prepare the full-length expression vectors of human Delta-1. The transformant is prepared by using expression plasmid containing DNA coding the thus constructed said human Delta-1.

As shown in Example 6, we have prepared expression vectors, which express extracellular proteins of human Serrate-1, as follows.

6) DNA coding the amino acids from No. 1 to 1036 in amino acid sequence in the sequence listing, SEQ ID NO: 6,
7) DNA coding chimera protein to which added polypeptide having FLAG sequence in the C-terminal of the amino acids from No. 1 to 1036 in amino acid sequence in the sequence listing, SEQ ID NO: 6, and
8) DNA coding chimera protein to which added said Fc sequence in the C-terminal of the amino acids from No. 1 to 1036 in amino acid sequence in the sequence listing, SEQ ID NO: 6, and to have dimer structure by disulfide bond in the hinge region,

are ligated individually with the expression vector pMKITNeo to prepare extracellular expression vectors of human Serrate-1.

The full-length expression vectors of the human Serrate-1 as the expression vectors, which express full-length proteins of the human Serrate-1, can be prepared as follows.

9) DNA coding amino acids from No. 1 to 1187 in the sequence listing, SEQ ID NO: 7 and
10) DNA coding chimera protein to which added polypeptide having FLAG sequence in the C-terminal of amino acids from No. 1 to 1187 in the sequence listing, SEQ ID NO: 7

are ligated individually with the expression vector pMKITNeo to prepare the full-length expression vectors of human Serrate-1. The transformant is prepared by using expression plasmid containing DNA coding the thus constructed said human Serrate-1.

Examples of the host are genus Escherichia, genus Bacillus, yeast and animal cells. Examples of animal cells are simian cell COS-7 and Vero, Chinese hamster cell CHO and silk worm cell SF9.

As shown in Example 7, the expression vectors of the above 1) - 10) are transduced individually; the human Delta-1 or human Serrate-1 are expressed in COS-7 cell (obtainable from the Institute of Physical and Chemical Research, Cell Development Bank, RCB0539), and the transformants which were transformed by these expression plasmids, can be obtained. Further, human Delta-1 polypeptide and human Serrate-1 polypeptide can be produced by culturing the transformants under preferable culture condition in medium by known culture method.

As shown in Example 8, human Delta-1 polypeptide and human Serrate-1 polypeptide can be isolated and purified from the above cultured mass, in general, by the following methods.

For extraction of the substance from cultured microbial cells or cells, microbial cells or cells are collected by known method such as centrifugation after the cultivation, suspended in preferable buffer solution, disrupted the microbial cells or cells by means of ultrasonication, lysozyme and/or freeze-thawing and collected crude extract by centrifugation or

filtration. The buffer solution may contain protein-denaturing agents such as urea and guanidine hydrochloride or surface active agents such as Triton-X. In case of secretion in the cultured solution, the cultured mass is separated by the known method such as centrifugation to separate from microbial cells or cells and the supernatant solution is collected.

The thus obtained human Delta-1 or human Serrate-1, which are contained in the cell extracts or cell supernatants, can be purified by known protein purification methods. During the purification process, for confirmation of existence of the protein, in case of the fused proteins of the above FLAG and human IgGFc, they can be detected by immunoassay using antibody against known antigen epitope and can be purified. In case of not to express as such the fused protein, the antibody in Example 9 can be used for detection.

Antibodies, which specifically recognize human Delta-1 and human Serrate-1, can be prepared as shown in Example 9. Antibodies can be prepared by the methods described in the reference (Antibodies a laboratory manual, E. Harlow et al., Cold Spring Harbor Laboratory) or recombinant antibodies expressed in cells by using immunoglobulin genes isolated by gene cloning method. The thus prepared antibodies can be used for purification of human Delta-1 and human Serrate-1. The human Delta-1 or human Serrate-1 can be detected and assayed by using antibodies which recognize specifically human Delta-1 or human Serrate-1 as shown in Example 9, and can be used for diagnostic agents for diseases accompanied with abnormal differentiation of cells such as malignant tumors.

More useful purification method is the affinity chromatography using antibody. Antibodies used in this case are antibodies described in Example 9. For fused protein, antibodies against FLAG in the case of FLAG, and protein G or protein A in the case of human IgGFc as shown in Example 8.

Any fused protein other than the protein as shown hereinabove can be used. For example, histidine Tag and myc-tag can be mentioned. Any fused proteins can be prepared by using methods of the present day's genetic engineering techniques other than the known methods, and peptides of the present invention derived from those fused proteins are in the scope of the present invention.

Physiological functions of the thus purified human Delta-1 and human Serrate-1 proteins can be identified by various assay methods, for example, physiological activity assaying methods using cell lines and animals such as mice and rats, assay methods of intracellular signal transduction based on molecular biological means binding with Notch receptor etc.

We have observed actions for blood undifferentiated cells by using IgG1 chimera proteins of human Delta-1 and human Serrate-1.

As a result, we have found that, as shown in Example 10, in the umbilical cord blood derived blood undifferentiated cells, in which CD34 positive cell fraction is concentrated, polypeptides of the present invention have suppressive action of colony forming action against blood undifferentiated cells, which shows colony formation in the presence of cytokines. The suppressive action is only observed in the presence of SCF. This kind of effect has never been known.

As shown in Example 11, we have found that a maintenance of colony forming cells is significantly extended by addition of IgG1 chimera protein of human Delta-1 or human Serrate-1 in the long term (8 weeks) liquid culture in the presence of cytokines such as SCF, IL-3, IL-6, GM-CSF and Epo. Further we have found that the polypeptides of the present invention had an action not to suppress growth of the colony forming cells. A cytokine, MIP-1α having migration and differentiation suppressive action of blood cells (Verfaillie et al., J. Exp. Med. 179, 643-649, 1994), has no action for maintaining undifferentiation for blood undifferentiated cells.

Further as shown in Example 12, we have found that as a result of adding IgG1 chimera protein of human Delta-1 or human Serrate-1 to the liquid culture in the presence of cytokines, the human Delta-1 and human Serrate-1 had activities for significantly maintaining LTC-IC (Long-Term Culture-Initiating Cells) number, which is positioned most undifferentiated blood stem cells in the human blood undifferentiated cells.

These results indicate that the human Delta-1 and human Serrate-1 suppress differentiation of blood undifferentiated cells, and these actions spread from blood stem cells to colony forming cells. These physiological actions are essential for in vitro expansion of blood undifferentiated cells. Cells cultured in the medium containing human Delta-1 or human Serrate-1 are efficient in recovery of suppresion of bone marrow after administration of anti tumor agents, accordingly in vitro growth of hemopoietic stem cells may be possible if other conditions would be completed. Further pharmaceuticals containing the polypeptide of the present invention have action protection and release of the bone marrow suppressive action, which is observed in adverse effects of antitumor agents.

Suppressive action for differentiation of cells in the undifferentiated cells other than blood cells is expected and stimulating action for tissue regeneration can be expected.

In the pharmaceutical use, polypeptides of the present invention are lyophilized with adding preferable stabilizing agents such as human serum albumin, and is used in dissolved or suspended condition with distilled water for injection when it is in use. For example, preparation for injection or infusion at the concentration of 0.1-1000 μg/ml may be provided. A mixture of the compound of the present invention 1 mg/ml and human serum albumin 1 mg/ml divided in a vial could maintain activity of the said compound for long term. For culturing and activating cells in vitro, lyophilized preparation or liquid preparation of the polypeptide of the present invention are prepared and are added to the medium or immobilized in the vessel for culture. Toxicity of the polypeptide of the present invention was tested. Any polypeptide,

10 mg/kg was administered intraperitoneally in mice, but no death of mice was observed.

In vitro physiological activity of the polypeptide of the present invention can be evaluated by administering to disease model mice or its resembled disease rats or monkeys, and examining recovery of physical and physiological functions and abnormal findings. For example, in case of searching abnormality in relation to hemopoietic cells, bone marrow suppressive model mice are prepared by administering 5-FU series of antitumor agents, and bone marrow cell counts, peripheral blood cell counts and physiological functions are examined in the administered group or the non administered group of mice. Further, in case of searching in vitro cultivation and growth of hemopoietic undifferentiated cells including hemopoietic stem cells, the bone marrow cells of mice are cultured in the groups with or without addition of the compound of the present invention, and the cultured cells are transferred into the lethal dose irradiated mice. Result of recovery is observed with the indications of survival rate and variation of blood counts. These results can be extrapolated to the humans, and accordingly useful effective data for evaluation of the pharmacological activities of the compound of the present invention can be obtained.

Applications of the compound of the present invention for pharmaceuticals include diseases with abnormal differentiation of cells, for example leukemia and malignant tumors. These are cell therapy, which is performed by culturing human derived cells in vitro with maintaining their original functions or adding new functions, and a therapy, which is performed by regenerating without damaged the functions of the originally existed in the tissues by administering the compound of the present invention under the regeneration after tissue injury. Amount of administration may differ in the type of preparation and is ranged from 10 μg/kg to 10 mg/kg.

Further strong physiological activity can be achieved by expression of forming multimer of the polypeptide of the present invention.

As shown in Example 10, since the suppressive action of human Delta-1 and human Serrate-1 is stronger in the IgG chimera protein having dimer structure, a form of stronger physiological activity is preferably expressed in the form of multimer formation.

Human Delta-1 and human Serrate-1 having multimer structure can be produced by a method of expressing chimera protein with human IgG Fc region as described in the example and expressing the multimer having disulfide bond with hinge region of the antibody, or a method expressing chimera protein, in which antibody recognition region is expressed in the C-terminal or N-terminal, and reacting with the polypeptide containing extracellular part of the thus expressed said human Serrate and the antibody which recognize specifically the antibody recognition region in the C-terminal or N-terminal. In the other methods, a method, in which a fused protein expressed with only the hinge region of the antibody and the dimerized by disulfide bond, can be mentioned. The multimer of human Delta-1 and human Serrate-1 having higher specific activity than the dimer can be obtained. The said multimer is constructed by fused protein which is prepared for expressing the peptide in the C-terminal, N-terminal or other region. The protein is prepared in the form of forming disulfide bond without effecting in any activities of the other human Delta-1 or human Serrate-1. The multimer structure can also be expressed by arranging one or more peptide, which is selected from polypeptides containing amino acids sequence of the sequence listing, SEQ ID NO: 2, 3, 5 or 6, with genetic engineering method in series or in parallel. Other known methods for providing multimer structure having dimer or more can be applied. Accordingly, the present invention includes any polypeptides containing amino acid sequences described in the sequence listing, SEQ ID NO: 2, 3, 5 or 6 in the form of dimer or more structure prepared by genetic engineering technique.

Further in the other method, multimerization method using chemical cross-linker can be mentioned. For example, dimethylsuberimidate dihydrochloride for cross-linking lysine residue. N-(γ-maleimidebutyryloxy) succinimide for cross-linking thiol group of cysteine residue and glutaraldehyde for cross-linking between amino groups can be mentioned. The multimer with dimer or more can be synthesized by applying these cross-linking reactions. Accordingly, the present invention includes any polypeptides containing amino acid sequences described in the sequence listing, SEQ ID NO: 2, 3, 5 or 6 in the form of dimer or more structure prepared by chemical cross-linking agents.

In application of medical care in which cells are proliferated and activated in vitro and are returned to the body, human Delta-1 or human Serrate-1 of the form hereinabove can be added directly in the medium, but immobilization can also be made. Immobilization method includes applying amino group or carboxyl group in the peptide, using suitable spacers or the above mentioned cross-linkers, and the polypeptide can be covalently bound to the culture vessels. Accordingly, the present invention includes any polypeptides containing amino acid sequences described in the sequence listing, SEQ ID NO: 2, 3, 5 or 6 in the form of existing on the solid surface.

Since the natural human Delta-1 and human Serrate-1 are cell membrane proteins, differentiation suppressive action in Example can be expressed by cocultivating with cells expressing these molecules and blood undifferentiated cells. Consequently, this invention includes cocultivation method with transformed cells by using DNA coding amino acid sequences in the sequence listing. SEQ ID NO: 2 - 7 and undifferentiated cells.

Expressed cell may be COS-7 cell as shown in Examples, but cells of human origin are preferable, and further expressed cells may be cell line or any of human in vivo blood cells and somatic cells. Consequently, the polypeptide can be expressed in vivo by integrated into vectors for gene therapy.

As shown in Example 10. FLAG chimera protein of human Delta-1 or human Serrate-1, both of which are low concentrated monomer, shows not a colony formation suppressive action but a colony formation stimulating action. This action may be involved in expressing Notch receptor and Notch ligand in the occasion of cell division of blood undifferentiated cells and acting the polypeptide of the present invention as an antagonist for that action. This suggests that the polypeptide having amino acid sequence of the sequence listing, SEQ ID NO: 1, 2, 4 or 5, shows colony formation stimulation action by controlling the concentration of its action.

This fact suggests that inhibition of binding the polypeptide having amino acid sequence in the sequence listing, SEQ ID NO: 2 - 7 and these receptors can be used for finding out molecules and compounds for stimulating cell differentiation. The methods include binding experiment using radio isotope, luciferase assay using transcriptional control factors, a down stream molecule of the Notch receptor, and simulation on the computer by X-ray structural analysis. Accordingly, the present invention includes screening method for pharmaceuticals using polypeptide in the sequence listing, SEQ ID NO: 2 - 7.

As shown in Example 13, specific leukemia cells can be differentiated by using IgG chimera protein of human Delta-1 or human Serrate-1. Consequently, the present invention can be applied for diagnostic reagents for leukemia or isolation of specific blood cells. This result indicates that human Delta-1 or human Serrate-1 molecule binds specifically with its receptor, a Notch receptor molecule. For example, expression of Notch receptor can be detected by using fused protein with the above extracellular region and human IgGFc. Notch is known to involve in some type of leukemia (Ellisen et al., Cell 66, 649-661, 1991). Accordingly, the polypeptide having amino acids sequence in the sequence listing, SEQ ID NO: 2, 3, 5 and 6 can be used for diagnostic reagents for in vitro or in vivo.

Brie explanation of the drawings

Fig. 1: Alignment of DSL domain of Notch ligand identified in various organisms including the molecules of the present invention.

Fig. 2: Suppression of colony formation of the blood undifferentiated cells using the molecules of the present invention.

Fig. 3: Concentration dependency of colony formation suppression of the blood undifferentiated cells using the molecules of the present invention.

Fig. 4: A graph showing calculation of LTC-1 after liquid culture using the molecules of the present invention.

Fig. 5: Cells stained by the molecules of the present invention.

Embodiments of the invention

Following examples illustrate the embodiments of the present invention but are not construed as limiting these examples.

Example 1

Cloning of PCR products using human Delta-1 primer and determination of base sequence

A mixed primer corresponding to amino acid sequence conserved in C-Delta-1 and X-Delta-1, i. e. sense primer DLTS1 (sequence listing, SEQ ID NO: 11) and antisense primer DLTA2 (sequence listing, SEQ ID NO: 12), were used.

A synthetic oligonucleotide was prepared by using automatic DNA synthesizer with the principle of immobilized method. The automatic DNA synthesizer used was 391PCR-MATE of Applied Biosystems Inc.,U.S.A. Nucleotide, carrier immobilized with 3' -nucleotide, solution and reagents are used according to the instructions by the same corporation. Oligonucleotide was isolated from the carrier after finishing the designated coupling reaction and treating the oligonucleotide carrier, from which protective group of 5'-teminal was removed, with concentrated liquid ammonia at room temperature for one hour. For removing the protective groups of nucleic acid and phosphoric acid, the reactant solution containing nucleic acid was allowed to stand in the concentrated ammonium solution in the sealed vial at 55°C for over 14 hours. Each oligonucleotide, from which the carrier and protective groups were removed, was purified by using OPC cartridge of the Applied Biosystems Inc., and detritylated by using 2 % trifluoracetic acid. Primer was dissolved in deionized water to set final concentration of 100 pmol/μ1 after purification.

Amplification of these primers by PCR was performed as follows. Human fetal brain originated cDNA mixed solution (QUICK-Clone cDNA, CLONTECH Inc., U.S.A.) 1 μ1 was used. 10 × buffer solution [500 mM KCl, 100 mM Tris-HCl (pH 8. 3), 15 mM $MgCl_2$, 0.01 % gelatin] 5 μ1, dNTP mixture (Takara Shuzo Co., Japan) 4 μ1, sense primer DLTS1 (100 pmo1/μ1) 5 μ1 which was specific to the above vertebrates and antisense primer DLTA2 (100 pmol/μ1) 5 μ1 and TaqDNA polymerase (AmpliTaq, Takara Shuzo Co., Japan, 5 U/μl) 0.2 μl were added thereto, and finally deionized water was added to set up total 50 μ1. PCR was performed by 5 cycles of a cycle consisting of treatment at 95°C for 45

seconds, at 42 °C for 45 seconds and 72°C for 2 minutes, further 35 cycles of a cycle consisting of treatment at 95 °C for 45 seconds, at 50 °C for 45 seconds and 72°C for 2 minutes, and finally allowed to stand at 72°C for 7 minutes. A part of the PCR products was subjected to 2 % agarose gel electrophoresis, stained with ethidium bromide (Nippon Gene Co., Japan), and observed under ultraviolet light to confirm amplification of about 400 bp DNA.

Total amount of PCR product was subjected to electrophoresis with 2 % agarose gel prepared by low melting point agarose (GIBCO BRL Inc., U.S.A.), stained by ethidium bromide, cutting out about 400 bp bands of PCR products by the Delta primer under the UV light, adding distilled water of the same volume of the gel, heating at 65°C for 10 minutes, and completely dissolving the gel. The dissolved gel was centrifuged at 15000 rpm for 5 minutes to separate supernatant solution after adding equal volume of TE saturated phenol (Nippon Gene Co., Japan) and the same separation operation was performed after adding TE saturated phenol : chloroform (1 : 1) solution and chloroform. DNA was recovered from the final solution by ethanol precipitation.

A vector, pCRII vector (Invitorogen Inc.,U.S.A., hereinafter designates as pCRII) was used. The vector and the above DNA in molar ratio of 1 : 3 were mixed and DNA was ligated into the vector by using T4 DNA ligase (Invitorogen Inc., U.S.A.). The pCRII, to which DNA was integrated, was subjected to gene transduction into E. coli one shot competent cells (Invitorogen Inc., U.S.A.) and was spread on the semi-solid medium plate of L-Broth (Takara Shuzo Co., Japan) containing ampicillin (Sigma Corp., U.S.A.) 50 μg/ml and allowed to stand at 37°C for about 12 hours. The appeared colonies were randomly selected, inoculated in the L-Broth liquid medium 2 ml containing same concentration of ampicillin and shake cultured at 37°C for about 18 hours. The cultured bacterial cells were recovered and the plasmid was separated by using Wizard Miniprep (Promega Inc., U.S.A.) according to the attached explanation sheet. The plasmid was digested by restriction enzyme EcoRI. Integration of the said PCR product was confirmed by incision of about 400 bp DNA. Base sequence of the incorporated DNA in the confirmed clone was determined by the fluorescent DNA sequencer (Model 373S, Applied System Inc., U.S.A.)

Example 2

Cloning of full length novel human Delta-1 and its analysis

A screening of clones having full length cDNA was performed by hybridization from human placenta origin cDNA library (inserted cDNA in λgt-11, CLONTECH Inc., U.S.A.) in plaques corresponding to 1×10^6 plaques. Generated plaques were transfered onto nylon filter (Hybond N+: Amersham Inc., U.S.A.). The transcribed nylon filter was subjected to alkaline treatment [allow to stand for 7 minutes on the filter paper permeated with a mixture of 1.5 M NaCl and 0.5 M NaOH], followed by twice neutralizing treatments [allow to stand for 3 minutes on the filter paper permeated with a mixture of 1.5 M NaCl, 0.5 M Tris-HCl (pH 7.2) and 1 mM EDTA]. Subsequently, the filter was shaken for 5 minutes in the 2-fold concentrated SSPE solution [0.36 M NaCl, 0.02 M sodium phosphate (pH 7.7) and 2 mM EDTA], washed and air-dried. Then the filter was allowed to stand for 20 minutes on the filter paper, which was permeated with 0.4 M NaOH, shaken for 5 minutes with 5-fold concentrated SSPE solution and washed, then again air-dried. Screening was conducted in the human Delta-1 probe labeled with radioisotope ^{32}P using these filters.

DNA probe prepared in Example 1 was labeled with ^{32}P as follows. A DNA fragment was cutted out by EcoRI from pCRII, inserted a purified PCR product (about 400 bp) by human Delta-1 primer and determined gene sequence, and was isolated from low melting point agarose gel. The thus obtained DNA fragment was labeled by DNA labeling kit (Megaprime DNA labeling system : Amersham, U.S.A.). The primer solution 5 μl and deionized water were added to DNA 25 ng to set up total volume of 33 μl, which was treated for 5 minutes in boiling water bath. Reaction buffer solution 10 μl containing dNTP, α - ^{32}P-dCTP 5 μl and T4 DNA polynucleotide kinase solution 2 μl were added thereto, treated at 37 °C for 10 minutes in water bath. Subsequently, the mixture was purified by Sephadex column (Quick Spin Column Sephadex G-50 : Boehringer Mannheim Inc., Germany), then treated for 5 minutes in boiling water bath and ice-cooled for 2 minutes for use.

Hybridization was performed as follows. The prepared filter hereinabove was immersed into the prehybridization solution consisting of SSPE solution, in which final concentration of each component is set at 5-fold concentration, 5-fold concentration of Denhardt's solution (Wako Pure Chemicals, Japan), 0.5 %SDS (sodium dodecyl sulfate, Wako Pure Chemicals, Japan) and salmon sperm DNA (Sigma, U.S.A.) 10 μg/ml denatured by boiling water, and shaken at 65°C for 2hours, then the filter was immersed into the hybridization solution of the same composition with the above prehybridization solution with the ^{32}P-labeled probe above mentioned and shaken at 65°C for 2 hours for 16 hours to perform hybridization.

The filter was immersed into SSPE solution containing 0.1 % SDS, shaken at 55°C and washed twice, further immersed into 10-fold dilution of SSPE solution containing 0.1% SDS and washed four times at 55°C. An autoradiography of the washed filter was performed using intensified screen. Clones of strongly exposed part were collected and the plaques obtained were again spread and screened by the same method hereinbefore to separate complete single clones.

The thus isolated phage clones were seven clones. Phage of all of these clones was prepared to about 1×10^9 pfu, purified the phage DNA, digested by restriction enzyme EcoRI and inserted into pBluescript (Stratagene Inc., U.S.A.) which was digested EcoRI in the same way. DNA sequences of the both ends of these clones were analyzed by DNA sequencer. Three clones of D5, D6 and D7 were the clone containing DNA sequence from No. 1 to 2244 in the sequence listing, SEQ ID NO: 8. A clone D4 was a clone containing DNA sequence from No. 999 to 2663 in the sequence listing, SEQ ID NO: 8. The clones D5 and D4 prepared the deletion mutant by using kilosequence deletion kit (Takara Shuzo Co., Japan) according to a description of the attached paper. Full-length cDNA base sequence of the present invention was determined using the DNA sequencer from both direction of 5' -direction and 3' -direction.

By applying with XhoI site at No. 1214 in DNA sequence in the sequence listing, SEQ ID NO: 8, D4 and D5 were digested by restriction enzyme XhoI to prepare plasmid pBSDel-1 containing full length of DNA sequence in the sequence listing, SEQ ID NO: 7.

Example 3

Cloning of human Serrate-1 specific PCR product and determination of base sequence

A mixed primer, which corresponded to amino acid sequence conserved in Drosophila Serrate and rat Jagged, i.e. sense primer SRTS 1 (the sequence listing, SEQ ID NO: 13) and antisense primer SRTA2 (the sequence listing, SEQ ID NO: 14), was used. Preparation was conducted by the same way as described in Example 1.

Amplification by PCR using these primers was performed as follows. To the human fetal brain originated cDNA mixed solution hereinbefore 1 μ1 was added 10× buffer solution (described in Example 1) 5 μl, said dNTP mixture 4 μ1, sense primer SRTS1 (100 pmol/μ1) 5 μ1 and antisense primer SRTA2 (100 pmol/ μl) 5 μl specific to Serrate-1 homologue hereinbefore, and said TaqDNA polymerase 0.2 μ1, and finally added deionized water to set up total volume 50 μl. The mixture was treated for 5 cyeles of a cycle consisting of at 95°C for 45 seconds, at 42°C for 45 seconds and 72°C for 2 minutes, and 35 cycles of a cycle onsisting of at 95°C for 45 seconds, at 50°C for 45 seconds and 72°C for 2 minutes, and finally allowed to stand at 72°C for 7 minutes to perform PCR. A part of the PCR product was subjected to 2 % agarose gel elctrophoresis, stained by ethidium bromide, and observed under ultraviolet light to confirm amplification of about 500 bp cDNA.

Total amount of PCR product was subjected to electrophoresis with 2 % agarose gel prepared by low melting point agarose, stained by ethidium bromide, cutting out about 500 bp bands under the UV light, adding distilled water of the equal volume of the gel, heating at 65°C for 10 minutes, and completely dissolving the gel. The dissolved gel was centrifuged at 15000 rpm for 5 minutes to separate supernatant solution after adding equal volume of TE saturated phenol and the same separation operation was performed after adding TE saturated phenol : chloroform (1 : 1) solution and chloroform. DNA was recovered from the final solution by ethanol precipitation.

A vector, pCRII vector was used. The vector and the above DNA were mixed in molar ratio of 1 : 3 and DNA fragment was ligated into the vector pCRII by the same method in Example 1. The pCRII, to which DNA was integrated, was subjected to gene transduction into E. coli. The appeared colonies were randomly selected and were inoculated in liquid medium L-Broth 2 ml containing the same concentration of ampicillin and shake cultured at 37°C for about 18 hours. The cultured bacterial cells were recovered and the plasmid was separated by using the Wizard Mini prep according to the attached explanatory sheet. The plasmid was digested by restriction enzyme EcoRI. Integration of the said PCR product was confirmed by incision of about 500 bp DNA. Base sequence of the incorporated DNA in the confirmed clone was determined by the fluorescent DNA sequencer.

Example 4

Cloning of full length novel human Serrate-1 and its analysis

A screening of clones having full length cDNA was performed by hybridization from the human placenta origin cDNA library hereinbefore in plaques corresponding to 1×10^6 plaques. Preparation of the filter was performed by the same method as described in Example 2. Screening was conducted in the human Serrate-1 probe labeled with radioisotope ^{32}P using the filter.

The above DNA probe labeled with ^{32}P was prepared by a method described in Example 2, and hybridization, washing of the filter and isolation of the clone were performed by the description in Example 2.

The thus isolated phage clones were 22 clones. Phage of all of these clones was prepared to about 1×10^9 pfu, purified the phage DNA, digested by restriction enzyme EcoRI and inserted into pBluescript which was digested EcoRI in the same way. DNA sequences of the both ends of these clones were analyzed by DNA sequencer. Two clones of SI6 and S20 were the clone containing DNA sequence from No. 1 to 1873 in the sequence listing, SEQ ID NO: 9. Two clones S5 and S14 were the clones containing DNA sequence from No. 990 to 4005 in the sequence listing, SEQ ID

NO: 9. These clones prepared the deletion mutants by using the kilosequence deletion kit according to a description of the attached leaflet. The cDNA base sequence coding the polypeptide of the present invention was determined using the DNA sequencer from both direction of 5'-direction and 3'-direction.

By applying with BglII site at No. 1293 in DNA sequence in the sequence listing, SEQ ID NO: 9, S20 and S5 were digested by restriction enzyme BglII, and DNA of gene sequence from No.1 to 4005 in the sequence listing SEQ ID NO: 9 was subcloned in E.coli vector pBluescript. This plasmid is named as pBSSRT.

Since the termination codon was not found in the C-terminal and the intracellular region coding C-terminal amino acids was not cloned, cloning of the full length gene was performed using the 3' RACE system kit, GIBCO-BRL, U.S.A., according to the description of the attached leaflet. The cloning of cDNA gene for 3'-direction was performed in polyA$^+$ RNA (CLONTECH Inc.,U.S.A.) originated from human placenta to determine the gene sequence.

The thus cloned three gene fragments by applying with BglII site in DNA sequence No. 1293 and AccI site in DNA sequence No. 3943 and a plasmid containing full length of DNA sequence in the sequence listing, SEQ ID NO: 5 were inserted between EcoRI and XbaI in the multi-cloning site of pUC18 to prepare pUCSR-1 containing full length gene of human Serrate-1. This gene sequence as well as its amino acid sequence is shown in the sequence listing, SEQ ID NO: 9.

Example 5

Preparation of expression vectors of human Delta-1

Using the gene consisting of DNA sequence described in the sequence listing, SEQ ID NO: 7, expression vectors of human Delta-1 protein mentioned in the following 1) - 5) were prepared. Addition of restriction enzyme sites and insertion of short gene sequence were performed using ExSite PCR-Based Site-Directed Mutagenesis Kit (Stratagene Inc.. U.S.A.) according to the operating manual.

1) Expression vector of soluble human Delta-1 protein (HDEX)

The cDNA coding polypeptide of amino acid sequence form No. 1 to 520 in the sequence listing, SEQ ID NO: 3 was ligated with expression vector pMKITNeo containing SRα promoter and neomycin resistance gene to prepare expression vector.

For preparation of expression vector of human Delta-1, in order to stable expression from gene product, EcoRI site was added in the 20 bp upper stream for 5'-direction of the initiation codon (gene sequence No. 179 in the sequence listing, SEQ ID NO: 8). Using the above Mutagenesis Kit, a plasmid pBSDel-1, which contained DNA sequence in sequence listing, SEQ ID NO: 8 and full length cDNA of human Delta-1 were set as the template, and oligonucleotides having gene sequence in sequence listing, SEQ ID NO: 15 and SEQ ID NO: 16 was set as the primers. Then DNA adding EcoRI site in the 20 bp upper stream for 5' -direction was prepared. Hereinafter this plasmid is designated as pBS/Eco-Delta.

The pBS/Eco-Delta was used as a template. In order to add the termination codon and restriction enzyme MluI site after a C-terminal position, using the Mutagenesis Kit, and setting oligonucleotides having gene sequences in the sequence listing, SEQ ID NO: 17 and SEQ ID NO: 18 as primers, addition of the termination codon and MluI site were performed. The resulted vector was digested by EcoRI and MluI, and about 1600 bp splitted gene fragment was ligated in pMKITNeo, which was treated by the same restriction enzyme, to construct the expression vector. This vector was designated as pHDEX.

2) Expression vector of FLAG chimera protein of soluble human Delta-1 (HDEXFLAG)

The cDNA coding chimera protein, to which cDNA coding FLAG sequence was added to the C-terminal of polypeptide from No. 1 to 520 of amino acid sequence in the sequence listing, SEQ ID NO: 3, was ligated to the expression vector pMKITNeo containing SRα promoter and neomycin resistance gene to prepare the expression vector.

Using pBS/Eco-Delta as template, FLAG sequence was added in the extracellular C-terminal, i.e. after Gly at No. 520 in the sequence listing, SEQ ID NO: 3. In order to add the termination condon and restriction enzyme MluI site, using the Mutagenesis Kit, and setting oligonucleotides having gene sequence in the sequence listing, SEQ ID NO: 19 and SEQ ID NO: 18 as primers, a gene coding FLAG sequence and termination codon and MluI site were added in the C-terminal. This vector was digested by EcoRI and MluI, and about 1700 bp splitted gene fragment was ligated to the similarly restriction enzyme treated pMKITNeo to construct the expression vector. This vector was designated as pHDEXFLAG.

3) Expression vector of IgG1Fc chimera protein of soluble human Delta-1 (HDEXIg)

A gene sequence coding polypeptide, to which amino acid sequence of Fc region below the hinge part of human IgG1 was added to the C-terminal of polypeptide having amino acid sequence in the sequence listing, SEQ ID NO: 3.

Preparation of fused protein with immunoglobulin Fc protein was performed according to the method of Zettlmeissl et al. (Zettlmeissl et al., DNA cell Biol., 9, 347-354, 1990). A gene using genome DNA with intron was applied and the said gene was prepared by using PCR. Human genome was used as a template. An oligonucleotide of the sequence in the sequence listing, SEQ ID NO: 20 with restriction enzyme BamHI site and an oligonucleotide of the sequence in the sequence listing, SEQ ID NO: 21 with restriction enzyme XbaI site were used as primers. PCR was performed using the primers and human genomic DNA as template. About 1.4 kbp band was purified, treated by restriction enzyme BamHI and XbaI (Takara Shuzo Co., Japan), and genes were ligated to pBluescript, which was similarly treated by restriction enzyme, by using T4 DNA ligase to prepare subcloning. Later, the plasmid DNA was purified and sequenced to confirm gene sequence, then the said gene sequence was confirmed as genomic DNA in the hinge region of heavy chain of the human IgG1, (The sequence is referred to Kabat et al., Sequence of Immunological Interest, NIH Publication No. 91 - 3242, 1991). Hereinafter this plasmid is designated as pBShIgFc.

Using the said pBS/Eco-Delta as template, and using the Mutagenesis Kit, restriction enzyme BamHI site was added in the extracellular C-terminal, i.e. after Gly at No. 520 in the sequence listing, SEQ ID NO: 3. Furthermore, in order to add restriction enzyme XbaI and MluI sites to the downstream, and setting the oligonucleotides having gene sequence in the sequence listing, SEQ ID NO: 22 and SEQ ID NO: 23, using the Mutagenesis Kit, BamHI, XbaI and MluI sites were added. This vector digested by XbaI and BamHI and about 1200 bp of gene fragment digested from the above pBShIgFc by XbaI and BamHI were ligated to prepare vector containing gene fragments coding the final objective soluble human Delta-1 IgGIFc chimera protein. Finally, this vector was digested by EcoRI and MluI and about 3000 bp splitted gene fragments were ligated with the similarly restriction enzyme treated pMKITNeo to construct the expression vector. This vector was designated as pHDEXIg.

4) Expression vector of full length human Delta-1 protein (HDF)

The cDNA coding polypeptide from No. 1 to 702 of amino acid sequence in the sequence listing, SEQ ID NO: 4, was ligated to the expression vector pMKITNeo containing SRα promoter and neomycin resistance gene to prepare the expression vector.

In order to add the termination codon in C-terminal of the full length sequence, i.e. after Val at No. 702 in the sequence listing, SEQ ID NO: 4 and restriction enzyme MluI site, using the Mutagenesis Kit and pBS/Eco-Delta as template and setting oligonucleotides having gene sequencein the sequence listing, SEQ ID NO: 24 and SEQ ID NO: 25 as primers, the termination codon and MluI site were added in the C-terminal. This vector was digested by EcoRI and MluI, and about 2200 bp splitted gene fragment was ligated to the similarly restriction enzyme treated pMKITNeo to construct the expression vector. This vector was designated as pHDF.

5) Expression vector of FLAG chimera protein (HDFLAG) of full length human Delta-1

The cDNA coding chimera protein, to which cDNA coding FLAG sequence was added to the C-terminal of polypeptide from No. 1 to 702 of amino acid sequence in the sequence listing, SEQ ID NO: 4, was ligated to the expression vector pMKITNeo containing SRα promoter and neomycin resistance gene to prepare the expression vector.

In order to add FLAG sequence in the C-terminal, the termination codon and restriction enzyme MluI site, setting oligonucleotides having gene sequence in the sequence listing, SEQ ID NO: 26 and SEQ ID NO: 25 as primers and using pBS/Eco-Delta as template, a gene coding FLAG sequence and termination codon and M1uI site were added in the C-terminal. From this vector, DNA coding full length of human Delta-1 was cloned in E. coli vector pUC19 to prepare vector pUCDL-1F coding full length of human Delta-1. This vector was digested by EcoRI and MluI, and about 2200 bp splitted gene fragments were ligated to the similarly restriction enzyme treated pMKITNeo to construct the expression vector. This vector was designated as pHDFLAG.

Example 6

Preparation of expression vectors of human Serrate-1

Using the gene consisting of DNA sequence described in the sequence listing, SEQ ID NO: 9, expression vectors of human Serrate-1 protein mentioned in the following 6) - 10) were prepared. Addition of restriction enzyme sites and insertion of short gene sequence were performed by using the ExSite PCR-Based Site-Directed Mutagenesis Kit as well as according to the operating manual.

6) Expression vector of soluble human Serrate-1 protein (HSEX)

The cDNA coding polypeptide of amino acid sequence form No. 1 to 1036 in the sequence listing, SEQ ID NO: 6 was ligated with expression vector pMKITNeo to prepare expression vector.

For preparation of expression vector of polypeptide expression cells having amino acid sequence from No. 1 to 1036 in the sequence listing, SEQ ID NO: 6, in order to express gene product more stable, EcoRI site was added in the 10 bp upper stream region for 5' - direction of the initiation codon (gene sequence No. 409 in the sequence listing. SEQ ID NO: 9). Using the above Mutagenesis Kit, a plasmid pBSSRT, which contained cDNA of human Serrate-1 from No. 1 to 4005 of DNA sequence in the sequence listing, SEQ ID NO: 9, was set as the template, and oligonucleotide having gene sequence in sequence listing, SEQ ID NO: 27 and oligonucleotide having gene sequence in sequence listing, SEQ ID NO: 28 were set as the primers. Then DNA adding EcoRI site in the I0 bp upper stream for 5' - direction was prepared.

The thus prepared vector (hereinafter designates as pBS/Eco-Serrate-1) was used as a template. In order to add the termination codon and further restriction enzyme MluI site in the extracellular C-terminal region, i.e. C-terminal of polypeptide in the sequence listing, SEQ ID NO: 6, using the Mutagenesis Kit, and setting oligonucleotide having gene sequence in the sequence listing, SEQ ID NO: 29 and oligonucleotide having gene sequence in the sequence listing, SEQ ID NO: 30 as primers, the termination codon and MluI site were added. The resulted vector was digested by EcoRI and MluI, and about 3200 bp splitted gene fragment was ligated in pMKITNeo, which was treated by the same restriction enzyme, to construct the expression vector. This vector was designated as pHSEX.

7) Expression vector of FLAG chimera protein of soluble human Serrate-1 (HSEXFLAG)

The cDNA coding FLAG chimera protein, which had FLAG sequence in the C-terminal of polypeptide from No. 1 to 1036 of amino acid sequence in the sequence listing, SEQ ID NO: 6, was ligated to the expression vector pMKITNeo containing SRα promoter and neomycin resistance gene to prepare the expression vector.

Using pBS/Eco-Serrate-1 as a template, FLAG sequence was added in the extracellular C-terminal, i.e. the C-terminal of polypeptide in the sequence listing, SEQ ID NO: 6. In order to add the termination codon and further restriction enzyme MluI site, using the Mutagenesis Kit, and setting oligonucleotide having gene sequence in the sequence listing, SEQ ID NO: 31 and oligonucleotide having gene sequence in the sequence listing, SEQ ID NO: 30 as primers, a gene coding FLAG sequence and termination codon and MluI site were added in the C-terminal. This vector was digested by EcoRI and MluI, and about 3200 bp splitted gene fragment was ligated to the similarly restriction enzyme treated pMKITNeo to construct the exprssion vector, This vector was designated as pHSEXFLAG.

8) Expression vector of IgG1Fc chimera protein of soluble human Serrate-1 (HSEXIg)

A gene sequence coding polypeptide, to which amino acid sequence of Fc region below the hinge part of human IgG1 was added to the C-terminal of polypeptide having amino acid sequence in the sequence listing, SEQ ID NO: 6.

In order to add restriction enzyme BamHI site in the extracellular C-terminal, i.e. after the polypeptide having the sequence in the sequence listing, SEQ ID NO: 6 and further restriction enzyme XbaI and MluI sites to its downstream, BamHI, XbaI and MluI sites were added Using pBS/Eco-Serrate-1 as a template by the Mutagenesis Kit, using oligonucleotide having gene sequence in the sequence listing, SEQ ID NO: 32 and oligonucleotide having gene sequence in the sequence listing, SEQ ID NO: 33, as primers. This vector digested by XbaI and BamHI and about 1200 bp of gene fragment digested from the above pBShIgFc by XbaI and BamHI were ligated to finally prepare a vector, which contained gene fragments coding IgG1Fc chimera protein of the soluble human Serrate-1. Finally, this vector was digested by EcoRI and MluI, and splitted about 4400 bp gene fragment was ligated to pMKITNeo to construct the expression vector. This vector was designated as pHSEXIg.

9) Expression vector of full length human Serrate-1 protein (HSF)

The cDNA coding polypeptide from No. 1 to 1187 of amino acid sequence in the sequence listing, SEQ ID NO: 7 was ligated with expression vector pMKITNeo containing SRα promoter and neomycin resistance gene to prepare expression vector.

For preparation of the full length expression vector about 900 bp splitted gene fragment from pBS/Eco-Serrate-1 digested by restriction enzyme EcoRI and BglII, and pUCSR-1 digested by the same restriction enzyme were ligated, and a vector pUC/Eco-Serrate-1 coding full length gene of human Serrate-1 was prepared.

In order to add the termination codon to the site after Val at No. 1187 in the sequence listing SEQ ID NO: 7, and further add the restriction enzyme MluI site, using the Mutagenesis Kit, the termination codon and MluI site were added to the C-terminal using oligonucleotides having gene sequence in the sequence listing, SEQ ID NO: 34 and SEQ ID

NO: 35 as primers and the pBS/Eco-Serrate-1 as a template. The resulted vector was digested by EcoRI and MuII, and about 3700 bp splitted gene fragments were ligated in pMKITNeo, which was treated by the same restriction enzyme, to construct the expression vector. This vector was designated as pHSF.

10) Expression vector of FLAG chimera protein of full length human Serrate-1 (HSFLAG)

The cDNA coding chimera protein, to which cDNA coding FLAG sequence was added in the C-terminal of polypeptide from No. 1 to 1187 of amino acid sequence in the sequence listing, SEQ ID NO: 7, was ligated to the expression vector pMKITNeo containing SRα promoter and neomycin resistance gene to prepare the expression vector.

In order to add FLAG sequence in the C-terminal the termination codon and further restriction enzyme MluI site, setting oligonucleotides having gene sequence in the sequence listing, SEQ ID NO: 36 and SEQ ID NO: 35 as primers, using pBS/Eco-Serrate-1 as a template a gene coding FLAG sequence, the termination codon and the MluI site were added in the C-terminal as same as similar manner. This vector was digested by EcoRI and MluI, and about 3700 bp splitted gene fragments were ligated to the similarly restriction enzyme treated pMKITNeo to construct the expression vector. This vector was designated as pHSFLAG.

Example 7

Expression and Gene transfer of the expression vectors into cells

The expression vectors prepared in Examples 5 and 6 were transduced into COS-7 cell (obtained from RIKEN Cell Bank Physical and Chemical Research Institute, Japan, RCB0539).

Cell culture before gene transduction was performed by culturing in D-MEM (Dulbecco modified Eagle's medium, GIBCO-BRL Inc., U.S.A.) 10 % FCS. On a day before gene transduction, medium of cells was changed to set cell counts 5×10^5 cells/ml and cultured for overnight. On the day of gene transduction, cells were sedimented by centrifugation, centrifugally washed twice with PBS(-) and prepared the cells to 1×10^7 cells/ml in 1 mM $MgCl_2$ and PBS(-). Gene transfer was performed by electroporation using gene transduction device Gene-pulsar (Bio-Rad Inc., U.S.A.). The above cell suspension 500 µl was collected in the cell for electroporation (0.4 cm), added expression vector 20 µg, and allowed to stand in ice for 5 minutes. Electroporation was performed under the condition 3 µF, 450 V twice, during the twice electroporation cell mixture was allowed to stand at room temperature. After 5 minutes stayed in ice, cells were spread in the culture medium, diameter 10 cm previously added 10 ml of medium, and cultured at 37°C in 5 % carbon dioxide incubator.

Next day, the culture supernatant solution was removed, washed the cells adhered to the dish twice with PBS(-) 10 ml. In case of expression vector pHDEX, pHDEXFLAG, pHDEXIg, pHSEX, pHSEXFLAG,and pHSEXIg, serum-free D-MEM 10 ml was added and cultured for 7 days. Culture supernatant solution was recovered and was replaced the buffer to PBS(-) by Centricon 30 (Amicon Inc., U.S.A.) and simultaneously the solution was concentrated to 10-fold to obtain cell culture supernatant solution.

In case of pHDF, pHDFLAG, pHSF, and pHSFLAG, medium was changed by D-MEM containing 10 % FCS, and cultured further 3 days to prepare cell lysate. Thus, 2×10^6 cells were suspended in the cell lysis buffer [50 mM Hepes (pH 7.5), 1 % Triton X100, 10 % glycerol, 4 mM EDTA, 50 µg/ml Aprotinin, 100 µM Leupeptin, 25 µM Pepstatin A and 1 mM PMSF] 200 µ1, allowed to stand in ice for 20 minutes and centrifuged at 14000 rpm for 20 minutes to remove supernatant solution to obtain cell lysate.

Using there sample, expression of proteins were detected by Western blotting.

Concentrated cultured supermatants or cell lysates were subjected to SDS-PAGE using an electrophoresis tank and polyacrylamide gel for SDS-PAGE (gradient gel 5 - 15 %) (ACI Japan Inc., Japan) according to manifactures construction. Samples were prepared by treatment in boiling water for 5 min. with 2-mercaptoethanol (2-ME) for reduction, and non-reduced condition without taking the above treatment. As a markers Rainbow Marker (high molecular weight, Amersham Inc.) was used. Sample buffer solution and electrophoresis buffer were prepared with reference to the attached leaflet. When the SDS-PAGE was finished, acrylamide gel was transcribed to PVDF membrane filter (BioRad Inc., U.S.A.) using the Mini Trans Blot Cell (BioRad Inc.).

The thus prepared filter was shaken overnight at 4°C in the Blockace (Dainippon Pharm. Co.,Japan), TBS-T [20 mM Tris. 137 mM NaCl (pH 7.6) and 0.1 % Tween 20] to blocking. According to the explanation of the attached leaflet of ECL Western blotting detection system (Amersham Inc.,U.S.A.); in case that the objective protein was human Delta-1 origin, anti-human Delta-1 mouse monoclonal antibody described in Example 9 was used as primary antibody; in case that protein was human Serrate-1 origin, anti-human Serrate-1 mouse monoclonal antibody described in Example 9 was used as primary antibody; and in case that protein was FLAG chimera, anti-FLAG M2 mouse monoclonal antibody (Eastman Kodak, U.S.A.) was used as primary antibody, and peroxidase labeled anti-mouse Ig sheep antibodies (Amersham Inc., U.S.A.) was reacted. In case of IgG chimera, peroxidase labeled anti-human Ig sheep antibodies

(Amersham Inc., U.S.A.) was reacted.

Reaction time for antibodies was 1 hour at room temperature, and at an interval of each reaction, washing was performed by shaking in TBS-T at room temperature for 10 minutes for three times. After the final washing, the filter was immersed in the reaction solution of ECL-Western blotting detection system (Amersham Inc., U.S.A.) for 5 minutes, and wrapped in polyvinylidene chloride wrap to expose X-ray film.

As the result, in the sample with treatment of reduction, the bands showing protein obtained by transduction of pHDEX and pHDEXFLAG was detetcd about 65 kD ; protein obtained by transduction of pHDEXIg was detected about 95 kD, and protein obtained by transduction of pHDF and pHDFLAG was detected about 85 kD. In the non-reduced sample, the bands showing protein obtained by transduction of pHDEXIg was detected slightly smeared bands at 120 kD to 200 kD, mainly about 180 kD, which showed about 2-fold of the reduction stage, consequently, dimer was formed.

And also, in the sample with treatment of reduction, the bands showing protein obtained by transduction of pHSEX and pHSEXFLAG was detected about 140 kD ; protein obtained by transduction of pHSEXIg was detected about 170 kD, and protein obtained by transduction of pHSF and pHSFLAG was detected about 150 kD. In the non-reduced sample, the bands showing protein obtained by transduction of pHSEXIg was detected slightly smeared bands at 250 kD to 400 kD, mainly about 300 kD, which showed about 2-fold of the reduction stage, consequently, dimer was formed.

In these experiments, however cell lysate and cultured supernatant of COS-7 cells, to which pMKITNeo vector was transduced as a control was tested., no bands reacted against anti-human Delta-1 mouse monoclonal antibody, anti-human Serrate-1 mouse monoclonal antibody, anti-FLAG antibody, and anti-human Ig antibody were detected.

Therefore, this ten-expression vector can produce the objective polypeptides.

Example 8

Purification of soluble human Delta-1 and human Serrate-1 proteins of gene transduction cells

Cultured supernatant of COS-7 cells consisting of HDBXFLAG. HDBXIg, HSEXFLAG and HSEXIg, all of which expression was detected by a method in Example 7, were prepared in large scale, and each chimera protein was purified by affinity column.

In case of HDEXFLAG and HSEXFLAG, 2 liter of the cultured supernatant obtained by the method in Example 7 was passed through a column packed with Anti-FLAG M2 Affinity Gel (Eastman Kodak, U.S.A.). The chimera protein was adsorbed in a column by a reaction of affinity of anti-FLAG antibody of the gel and FLAG sequence of the chimera protein. Column, inner diameter 10 mm, disposable column (BioRad Inc.,U.S.A.) was used with packing the above gel 5 ml. A circulation system consisting of medium bottle → column → peristaltic pump → medium bottle was set up. The circulationwas run by a flow I ml/min. for 72 hours. Thereafter the colulnn was washed with PBS (-) 35 ml and eluted by 0.5 M Tris-glycine (pH 3.0) 50 ml. The eluate of 25 fractions, each 2 ml, was collected into the tube, and each fraction was neutralized by 200 μl of 0.5 M Tris-HC1 (pH 9.5) previously added in each tube.

The eluate fraction, each 10 μ1 of the secretor FLAG chimera protein which was purified by the above method was subjected to reduction treatment described in Example 7. SDS-PAGE electrophoresis by 5-10 % gradient polyacrylamide gel was performed. After finishing the electrophoresis, silver staining was conducted by using Wako silver stain kit II (Wako Pure Chemicals, Japan) according to the explanation of the attached leaflet. Fractions from No. 4 to 8 showed detectable bands in HSFLAG. The size is identical with the result of Western blotting of anti-FLAG antibody obtained in Example 6 in both of HDEXFLAG and HSEXFLAG. Therefore, purified HDEXFLAG and HSEFLAG were obtained.

In the IgG1Fc chimera protein, i. e. HDEXIg and HSEXIg, the cultured supernatant solution 2 liter was adsorbed in Protein A Sepharose colulnn (Pharmacia Inc., Sweden) according to the same method as of FLAG chimera protein to collect the eluate fractions. Using a part of eluate as same as in FLAG chimera protein, a determination of the eluate fraction, identification of the size and detection of the purity were performed by SDS-PAGE electrophoresis and silver stainlng in the reduced condition. Therefore, the eluate fraction from No. 4 to 15 were detected the bands. The size thereof is identical with the result of Western blotting using anti-human Ig antibody in both of HDEXIg and HSEXIg. Therefore, purified HDEXIg and HSEXIg were obtained.

Example 9

Preparation of antibodies recognizing human Delta-I and human Serrate-1

HDEXFLAG and HSEXFLAG, purified by the method in Example 8, were used as immunogen, and rabbits were immunized. After assaying antibody titer, whole blood was collected and serum was obtained. Anti-human Delta-I rabbit polyclonal antibody and anti-human Serrate-1 rabbit polyclonal antibody were purified by using the econopack serum IgG purification kit (BioRad Inc., U.S.A.) with reference to the attached explanation leaflet.

HDEXFLAG and HSEXFLAG purified by a method described in Example 8 were used as Immunogens, and mouse

monoclonal antibodies were prepared according to the explanation of the textbook. The purified HDEXFLAG or HSEXFLAG was administered in Balb/c mice (Nippon SLC CO., Japan) separately, 10 μg/mouse, immunized intracutaneously and subcutaneously. After second immunization, increased serum titer was confirmed by collecting blood ophthalmologically, the third immunization was performed. Subsequently, the spleen of mice was collected and fused with mouse myeloma cells P3 × 63Ag8 (ATCC TIB9) using polyethylene glycol. Hybridoma was selecled by HAT medium (Immunological and Biological Research Institute, Japan) , and the hybridoma strains which produced antibody specifically recognizing extracellular region of human Delta-1 or human Serrate-I in the medium, were isolated by enzyme immunoassay. The hybridoma strains producing mouse monoclonal antibody, which specifically recognized human Delta-1 or human Serrate-1, were established.

Anti-human Delta-1 monoclonal antibody and anti-human Serrate-1 monoclonal antibody were purified and prepared by using Mab Trap GII (Pharmacia Inc., Sweden) and according to the explanation of the leaflet, from the supenatant of the thus established hybridoma.

Affinity column was prepared by using these monoclonal antibodies. Preparation of the affinity column was performed according to the explanation attached to the CNBr activated Sephadex 4B (Pharmacia Inc., Sweden). A column. 2 cm^2 × I cm, containing gel 2 ml, was prepared.

A concentrated solution of the supernatant of the cultured COS-7 cells, to which pHDEX was gene transduced, was passed through the column for which anti-human Delta-1 monoclonal antibody was bound. A concentrated solution of the supernatant of the cultured COS-7 cells, to which pHSEX was gene transduced, was passed through the column, for which anti-human Serrate-1 monoclonal antibody was bound. Each supernatant solution was passed at 20 ml/hr, subsequently PBS (-) 15 ml was passed at the same flow rate and washed the column. Finally, the products were eluted by a mixture of 0.1 M sodium acetate and 0.5 M NaCl (pH 4.0). The eluate, each I ml fraction, was collected, and was neutralized by adding 1M Tris-HC1 (pH 9.1) 200 μl for each fraction.

SDS-PAGE of each purified protein was conducted under reduced condition according to the method described in Example 8, followed by silver staining and Western blotting to estimate molecular weight. HDEX, about 65 kD, was purified from concentrated supernatant of the cultured COS-7 cells, to which pHDEX was gene transduced, and HDSEX, about 140 kD, was purified from concentrated supernatant of the cultured COS-7 cells, to which pHSEX was gene transduced. Consequently, human Delta-I and human Serrate-1 can be purified by these affinity columns.

Example 10

Effects of HDEXIg and HSEXIg to colony formation of blood undifferentiated cells

In order to observe physiological action of HDEXIg and HSEXIg on blood undifferentiated cells, CD34 positive cells were cultured in the serum-free semi solid medium in the presence of HDEXIg and HSEXIg and known cytokines, and number of colony forming cells were observed.

Human umbilical cord blood or adult human normal bone marrow blood was treated by the silica solution (Immunological and Biological Research Institute, Japan) according to the attached explanation leaflet. Thereafter the low density cellular fraction (< 1.077 g/ml) was fractionated by densitometric centrifugation of Ficoll pack (Pharmacia Inc., Sweden) to prepare mononuclear cells. CD34 positive cells of human umbilical cord blood or human normal bone marrow blood was isolated from the mononuclear cells.

Separation of CD34 positive cells was performed by using Micro-Selector System (AIS Inc., U.S.A.) or Dynabeads M-450 CD34 and DETACHa-BEADS CD34 (Dynal Inc., Norway) according to attached explanation leaflets. After separation, the purity was measured as follows. Cells were stained by FITC labeled CD34 antibody HPCA2 (Beckton-Deckinson Inc., U.S.A.) and examined by a flow-cytometer (FACSCalibur, Beckton-Deckinson, U.S.A.). Purity above 85 % was confirmed for use.

The thus isolated CD34 positive cells were suspended homogeneously to form 400 cells/ml of the medium hereinbelow, and spread in the 35 mm dish (Falcon Inc.,U.S.A.), then cultured for 2 weeks in carbon dioxide incubator at 37°C under 5 % carbon dioxide, 5 % oxygen, 90 % nitrogen and 100 % humidity. The formed blood colonies were counted under the invert microscope.

A medium used is α-medium (GIBCO-BRL, U.S.A.), containing 2 % deionized bovine serum albumin (BSA, Sigma, U.S.A.), 10 μg /ml human insulin (Sigma, U.S.A.) 200 μg/ml transferrin (Sigma, U.S.A.), 10^{-5}M 2-mercaptoethanol (Nakarai Tesk Co., Japan), 160 μg/ml soybean lectin (Sigma, U.S.A.), 96 μg/ml cholesterol (Sigma, U.S.A.) and 0.9 % methylcellulose (Wako Pure Chemicals, Japan).

To the above medium under the following three conditions of cytokines, human Delta-I extracellular Ig chimera protein (HDEXIg) or human Serrate-I extracellular Ig chimera protein (HSEXIg) were added to the final concentration of 1 μg/ml. For control, human IgG1 (Ahens Research and Technology Inc., U.S.A.) was added with the same concentration in order to observe effect of IgGFc region.

Conditions of cytokines are as follows.

1 : 100 μg/ml, human SCF(Intergen Inc.,U.S.A.), 10 ng/ml humnan IL-3 (Intergen Inc.,U.S.A.). 100 ng/ml human IL-6 (Intergen Inc.,U.S.A.)
2 : 100 ng/ml human SCF, 10 ng/ml human IL-3, 4 ng/ml human thrombopoietin (Pepro Tech Inc.,U.S.A.)
3 : 100 ng/ml human SCF, 10 ng/ml human IL-3, 100 ng/ml human IL-6 , 2U/ml Epo (Chugai Seiyaku Co., Japan) 10 ng/ml human G-CSF (Chugai Seiyaku Co., Japan)

Results are shown in Fig. 2. In Fig. 2, A is a case of human Delta-I extracellular Ig chimera protein (HDEXIg), and B is a case of human Serrate-1 extracellular Ig chimera protein (HSEXIg). For A and B, each different origin human umbilical cord blood CD34 positive cell was used. The vertical axis : number of colonies. White : control, black : HDEXIg or HSEXIg. Both HDEXIg and HSEXIg have suppressive action of colony formation. No differences of the activities on the types of colonies were noted. Therefore, the molecular of the present invention has suppressive action for colony formation against colony forming cells of blood undifferentiated cells, i.e. diferentiation-suppressive action. Comparison with or without SCF on the activity indicated that the suppressive action tended to observe only in the presence of SCF.

Dose-dependent manner of the activity was studied. Comparison with dimer HSEXIg and monomer HSEXFLAG was performed. Result is shown in Fig. 3. Concentration in this case is indicated as molar concentration. For comparison with dimer and monomer, dimer HSEXIg was indicated by exact two molar concentrations and was plotted equivalent molar concentration of the human Serrate-1. Vertical axis indicates colony forming counts and horizontal axis indicates molar concentration. Colony forming counts without Notch ligand were plotted on the vertical axis in the zero concentration. For comparison, colony forming counts of human IgG1 1 μg/ml, was about 100 colonies.

This result indicated that HSEXIg and HSEXFLAG suppressed colony formation in dose-dependent manner. Activity of dimer HSEXIg was stronger than the monomer. A monomer HSEXFLAG showed stimulative action for colony formation in the low concentration area.

Example 11

Actions of HDEXIg and HSEXIg on long term liquid culture of colony forming blood undifferentiated cells

For observing physiological action of HDEXIg and HSEXIg on the blood undifferentiated cells, umbilical cord blood CD34 positive cells were culture for long term in the serum-free liquid medium in the presence of HDEXIg or HSEXIg and known cytokines, and numbers of colony forming cells were observed.

The umbilical cord blood mononuclear CD34 positive cells separated by a method described in Example 10 were liquid cultured at 1000 cells/well in the 24 well cell culture plate (Falcon Inc.,U.S.A.). Culture was performed at 37°C in the carbon dioxide incubator under 5 % carbon dioxide and 100 % humidity. Liquid culture medium was Iscove's modified Dulbecco's medium (IMDM, GIBCO-BRL, U.S.A.) added with 2 % BSA, 10 μg/ml human insulin, 200 μg/ml transferrin, 40 μg/ml low density lipoprotein (GIBCO-BRL, U.S.A.), 10^{-5}M 2-mercaptoethanol, 50 ng/ml human SCF, 5 ng/ml human IL-3, 10 ng/ml human IL-6, 5 ng/ml human GM-CSF (Intergen Inc., U.S.A.), and 3 U/ml Epo. If necessary condition, 500 ng HSIg or 50 ng/ml MIP-1 α (Intergen Inc.,U.S.A.) was added. The medium was added 1 ml/well and half of the medium was changed three times in a week. After culturing 2, 4, 6 and 8 weeks, all cells were collected from wells by using cell scraper in 1.5 ml micro tube. Cells were precipitated by centrifugation and resuspended in a fresh IMDM 1 ml, counted the cell counts by using hemocytometer, and in 5000 cells/m1. blood cell colony forming assay was performed.

Blood cell colony forming assay was performed using the Iscove' s methylcellulose complete ready mix (Stem Cell Technologies Inc., Canada), and each I ml was inoculated in two plates of 35 mm dish (Falcon Inc., U.S.A.) and incubated for 2 weeks in the carbon dioxide incubator. Blood colonies were counted CFU-GM and BFU-E in the invert microscope, and total was counted as CFU-C. CFU-C counts and cell counts obtained by hemocytometer were multiplied to obtain CFU-C count/1000 cells inoculated in the liquid culture.

In Table 1, result of HDEXIg and in Table 2, result of HSEXIg are shown. Experiments were conducted at n = 3, values obtained were shown by (mean ± SD). In the table, ND means no detection of colony.

Table 1

Colony forming cell maintenance action in the long-term liquid culture of human Delta-1 of the present invention			
Week	Cytokines		
	-	MIP-1 α	HDEXIg
0	69 ± 9	68 ± 9	68 ± 9
2	1440 ± 120	720 ± 110	1280 ± 230
4	340 ± 40	420 ± 80	410 ± 90
6	28 ± 6	96 ± 17	290 ± 60
8	ND	ND	88 ± 13

Table 2

Colony forming cell maintenance action in the long-term liquid culture of human Serrate-1 of the present invention			
Week	Cytokines		
	-	MIP-1 α	HSEXIg
0	68 ± 9	68 ± 9	68 ± 9
2	1440 ± 120	720 ± 110	1360 ± 280
4	340 ± 40	420 ± 80	560 ± 70
6	28 ± 6	96 ± 17	220 ± 50
8	ND	ND	130 ± 50

CFU-C could only be observed until 6th week of cultivation under the condition without cytokines for maintaining undifferentiated condition, and under the condition with MIP-1 α. It could be observed at 8th week in the presence of HDEXIg or HSEXIg. In comparison with MIP-1 α and HDEXIg and HSEXIg, MIP-1 α strongly suppressed colony formation at 2 weeks of culture, however no suppression in HDEXIg and HSEXIg were observed. In maintenance of CFU-C counts at 6 and 8 weeks of culture, HDBXIg and HSEXIg were superior.

Example 12

Effects of HDEXIg and HSEXIg on liquid culture of blood undifferentiated cell LTC-IC

In order to observing physiological action of HDEXIg and HSEXIg on the blood undifferentiated cells umbilical cord blood CD34 positive cells were cultured for two weeks in the serum-free liquid medium in the presence of HDEXIg or HSEX1g and known cytokines, and numbers of LTC-IC, which was thought to be most undifferentiated blood cells at present were observed.

The umbilical cord blood monocyte CD34 positive cells, 100000 to 20000 cells, separated by a method described in Example 10 were cultured in the following medium for 2 weeks. Numbers of LTC-IC in 4 experimental groups, which include a group before cultivation, a group of HDEXIg, a group of HSEXIg and a control group, were determined. Media used in liquid culture medium were α -medium added with 2 % BSA, 10 μg/ml human insulin, 200 μg/ml transferrin, 40 μg/ml low density lipoprotein, and 10^{-5}M 2-mercaptoethanol, further added with 100 ng/ml human SCF, 10 ng/ml human IL-3, and 100 ng/ml human IL-6. HDEXIg or HSEXIg 1 μg/ml were added to the above medium. In the control group, human IgG1 was added in the equal concentration.

Preparation of human bone marrow stromal cell layer used for LTC-IC, and quantitative assay of frequency of LTC-IC by a limit dilution were performed according to a method of Sutherland et al. (Blood. 74, 1563-, 1989 and Proc, Natl. Acad. Sci, USA, 87, 3584-, 1990) .

The bone marrow mononuclear cells, 1-2 × 10^7 cells, obtained in Example 10 before the separation and without the silica solution treatment, were cultured in LTC medium (MyeloCul, Stem Cell Technologies Inc., Canada) 5 ml added with hydrocortison 1 μM (Upjohn Japan Co., Japan) in T-25 flask (Falcon Inc., U.S.A,) at 37°C under 5 % carbon dioxide and 100 % humidity in the carbon dioxide incubator. Culture was conducted until the adhesive cell layers of the stromal cell formation spread more than 80 % of the bottom area of the culture. Detachment of the cell layer was performed by treating with EDTA solution (Cosmobio Co., Japan). Cells were plated in the 96 well plate (Beckton-Deckinson Inc.,U.S.A.), about 2 × 10^4 cells/well and re-cultivation was continued in the same medium. X-ray, 15Gy, 250 KV peak was irradiated after reconstituted stromal cell layer. Growth of stromal cells was stopped and blood cells in the stromal cells were removed by this treatment. The thus prepared stromal cells were used as stromal cell layer for the experiments.

In the assay of LTC-IC, cell counts in each group were adjusted within the ranges of 25-400 cells/well for CD34 positive cells before the cultivation, and 625-20000 cells/well for the cells after the cultivation, and cells were diluted for six step-dilution within these ranges. Each dilutes step of cells was co-cultured with the above stromal cell layer in the 96 well plate, for 16 wells/cells of one dilution step. Culture was performed in the same medium as used in stromal formation, at 37°C under 5 % carbon dioxide and 100 % humidity in the carbon dioxide gas incubator for 5 weeks. Cells in suspension and in attachment after cultivation were recovered in each well. Collected cells were transferred to the semi-solid culture medium consisting of α-medium added with 0.9 % methylcellulose, 30 % fetal calf serum (FCS, ICN Biomedical Japan), 1 % BSA, 10^{-5}M 2-mercaptoethanol, 100 ng/ml human SCF, 10 ng/ml human IL-3, 100 ng/ml human IL-6, 2U/ml Epo and 10 ng/ml human G-CSF. After 2 weeks of cultivation, colony forming cells were detected as the same was as described in Example 10 and 11, and numbers of well, in which colony forming cells were found, were detected. Incidence of LTC-IC was calculated according to the method of Taswell et al. (J. Immunol. 126, 1614-, 1981) based on the above data.

Graph used for calculation is shown in Fig. 4. In Fig. 4, calculation curves after liquid culture is shown. A vertical axis shows ratio of well for no colonies were observed, and a horizontal axis shows number of cells/well. In each experimental group, numbers of well, for which colonies were not observed, and numbers of cells were plotted, then regression curve was calculated by the least square method. Number of cells corresponding to number of 0.37 (a reciprocal of a base of natural logarithm) for which colonies did not appeared, was calculated. A reciprocal of that number of cells is a frequency of LTC-IC. Further, absolute number of LTC-IC was calculated from initial number of cells and frequency of LTC-IC.

Result indicated that 243 LTC-IC were found in 25000 cells before the liquid culture. In the control group number of cells during 2 weeks of cultivation increased to 1,510,000 cells, and LTC-IC was decreased to 49 cells. However, culturing with human Delta-1, i.e. HDEXIg or human Serrate-1, i.e. HSEXIg, numbers of cells were maintained in 1,310,000 and 1,140,000, respectively, and numbers of LTC-IC were slightly decreased to 115 and 53. Consequently, polypeptide of the present invention, especially human Delta-1 could have an activity for maintenance of number of LTC-IC in the liquid culture.

Example 13

Binding of HDEXIg and HSEXIg for blood cells

Binding of Notch ligands with various blood cells was studied using specific binding of Notch ligands to Notch receptors.

Blood cell lines tested were Jurkat (ATCC TIB-152), Namalwa (ATCC CRL-1432), HL-60 (ATCC CRL-1964), K562 (ATCC CCL-243), THO-1 (ATCC TIB-2 02), UT-7 (Komatsu et al., Cancer Res.,51, 341-348, 1991), Mo7e (Avanzi et al. Br. J.Haematol.,69, 359-, 1988) and CMK (Sato et al. Exp. Hematol., 15, 495-502, 1987). Culturing media for these cells were found in the reference or ATCC CELL LIMES & HYBRIDOMAS, 8th Ed, (1994).

Cells, 1 × 10^6 cells, were suspended in Hank' s balanced salt solution containing 2% FCS and 10 mM Hepes. HDEXIg or HSEXIg 1 μg/ml were added therein and allowed to stand at 4°C for overnight. Cells were washed twice with the Hank's solution. PE labeled sheep anti-human IgG monoclonal antibody 1 μg/ml was added, allow to stand in ice-cooling for 30 minutes, washed twice with the Hank' s solution, and suspended in the Hank' s solution 1 ml. Analysis was performed using the flow cytometer (FACSCalibur). Control groups were used with human IgG1 staining in place of HDEXIg or HSEXIg staining.

Results are shown in Fig. 5. A vertical axis indicates cell counts and a horizontal axis indicates fluorescence intensity. Staining with HDEXIg or HSEXIg is shown by solid line and control, a staining with human IgG1 is shown by a broken line. In Fig. 5, the left column shows HDEXIg and the right column shows HSEXIg. As shown in Fig. 5, results

indicate that Jurkat : reacted, Namalwa : non-reacted, HL-60 : non-reacted, K562 : non-reacted, THP-1 : non-reacted, UT-7 : reacted, Mo7e non-reacted and CMK : reacted. Since the same results in HDEXIg and HSEXIg were obtained, both recognized the identical molecule and these cells can be differentiated.

Effect of the invention

Notch ligand moleclue of the present invention can be used for maintenance of undifferentiated-suppressive substance, and pharmaceuticals.

SEQUENCE LISTING

INFORMATION FOR SEQ ID NO : 1

LENGTH : 43

TYPE : amino acid

TOPOLOGY : linear

MOLECULE TYPE : protein

SEQUENCE DESCRIPTION : SEQ ID NO : 1 :

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 1               5                  10                  15
Arg Asx Asp Xaa Phe Gly His Xaa Xaa Cys Xaa Xaa Xaa Gly Xaa Xaa
            20                  25                  30
Xaa Cys Xaa Xaa Gly Trp Xaa Gly Xaa Xaa Cys
        35                  40
```

INFORMATION FOR SEQ ID NO : 2

LENGTH : 200

TYPE : amino acid

TOPOLOGY : linear

MOLECULE TYPE : protein

ORIGINAL SOURCE

ORGANISM : human

SEQUENCE DESCRIPTION : SEQ ID NO : 2 :

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Ser Gly Val Phe Glu Leu Lys Leu Gln Glu Phe Val Asn Lys Lys Gly
 1               5                  10                  15
Leu Leu Gly Asn Arg Asn Cys Cys Arg Gly Gly Ala Gly Pro Pro Pro
            20                  25                  30
Cys Ala Cys Arg Thr Phe Phe Arg Val Cys Leu Lys His Tyr Gln Ala
        35                  40                  45
Ser Val Ser Pro Glu Pro Pro Cys Thr Tyr Gly Ser Ala Val Thr Pro
    50                  55                  60
Val Leu Gly Val Asp Ser Phe Ser Leu Pro Asp Gly Gly Gly Ala Asp
65                  70                  75                  80
```

Ser Ala Phe Ser Asn Pro Ile Arg Phe Pro Phe Gly Phe Thr Trp Pro
85 90 95
Gly Thr Phe Ser Leu Ile Ile Glu Ala Leu His Thr Asp Ser Pro Asp
100 105 110
Asp Leu Ala Thr Glu Asn Pro Glu Arg Leu Ile Ser Arg Leu Ala Thr
115 120 125
Gln Arg His Leu Thr Val Gly Glu Glu Trp Ser Gln Asp Leu His Ser
130 135 140
Ser Gly Arg Thr Asp Leu Lys Tyr Ser Tyr Arg Phe Val Cys Asp Glu
145 150 155 160
His Tyr Tyr Gly Glu Gly Cys Ser Val Phe Cys Arg Pro Arg Asp Asp
165 170 175
Ala Phe Gly His Phe Thr Cys Gly Glu Arg Gly Glu Lys Val Cys Asn
180 185 190
Pro Gly Trp Lys Gly Pro Tyr Cys
195 200

INFORMATION FOR SEQ ID NO : 3
LENGTH : 520
TYPE : amino acid
TOPOLOGY : linear
MOLECULE TYPE : protein
ORIGINAL SOURCE
ORGANISM : human
SEQUENCE DESCRIPTION : SEQ ID NO : 3 :

Ser Gly Val Phe Glu Leu Lys Leu Gln Glu Phe Val Asn Lys Lys Gly
1 5 10 15
Leu Leu Gly Asn Arg Asn Cys Cys Arg Gly Gly Ala Gly Pro Pro Pro
20 25 30
Cys Ala Cys Arg Thr Phe Phe Arg Val Cys Leu Lys His Tyr Gln Ala
35 40 45
Ser Val Ser Pro Glu Pro Pro Cys Thr Tyr Gly Ser Ala Val Thr Pro

50 55 60

Val Leu Gly Val Asp Ser Phe Ser Leu Pro Asp Gly Gly Gly Ala Asp

65 70 75 80

Ser Ala Phe Ser Asn Pro Ile Arg Phe Pro Phe Gly Phe Thr Trp Pro

85 90 95

Gly Thr Phe Ser Leu Ile Ile Glu Ala Leu His Thr Asp Ser Pro Asp

100 105 110

Asp Leu Ala Thr Glu Asn Pro Glu Arg Leu Ile Ser Arg Leu Ala Thr

115 120 125

Gln Arg His Leu Thr Val Gly Glu Glu Trp Ser Gln Asp Leu His Ser

130 135 140

Ser Gly Arg Thr Asp Leu Lys Tyr Ser Tyr Arg Phe Val Cys Asp Glu

145 150 155 160

His Tyr Tyr Gly Glu Gly Cys Ser Val Phe Cys Arg Pro Arg Asp Asp

165 170 175

Ala Phe Gly His Phe Thr Cys Gly Glu Arg Gly Glu Lys Val Cys Asn

180 185 190

Pro Gly Trp Lys Gly Pro Tyr Cys Thr Glu Pro Ile Cys Leu Pro Gly

195 200 205

Cys Asp Glu Gln His Gly Phe Cys Asp Lys Pro Gly Glu Cys Lys Cys

210 215 220

Arg Val Gly Trp Gln Gly Arg Tyr Cys Asp Glu Cys Ile Arg Tyr Pro

225 230 235 240

Gly Cys Leu His Gly Thr Cys Gln Gln Pro Trp Gln Cys Asn Cys Gln

245 250 255

Glu Gly Trp Gly Gly Leu Phe Cys Asn Gln Asp Leu Asn Tyr Cys Thr

260 265 270

His His Lys Pro Cys Lys Asn Gly Ala Thr Cys Thr Asn Thr Gly Gln

275 280 285

Gly Ser Tyr Thr Cys Ser Cys Arg Pro Gly Tyr Thr Gly Ala Thr Cys

290 295 300

Glu Leu Gly Ile Asp Glu Cys Asp Pro Ser Pro Cys Lys Asn Gly Gly

```
305                 310                 315                 320
Ser Cys Thr Asp Leu Glu Asn Ser Tyr Ser Cys Thr Cys Pro Pro Gly
                325                 330                 335
Phe Tyr Gly Lys Ile Cys Glu Leu Ser Ala Met Thr Cys Ala Asp Gly
            340                 345                 350
Pro Cys Phe Asn Gly Gly Arg Cys Ser Asp Ser Pro Asp Gly Gly Tyr
        355                 360                 365
Ser Cys Arg Cys Pro Val Gly Tyr Ser Gly Phe Asn Cys Glu Lys Lys
    370                 375                 380
Ile Asp Tyr Cys Ser Ser Ser Pro Cys Ser Asn Gly Ala Lys Cys Val
385                 390                 395                 400
Asp Leu Gly Asp Ala Tyr Leu Cys Arg Cys Gln Ala Gly Phe Ser Gly
                405                 410                 415
Arg His Cys Asp Asp Asn Val Asp Asp Cys Ala Ser Ser Pro Cys Ala
            420                 425                 430
Asn Gly Gly Thr Cys Arg Asp Gly Val Asn Asp Phe Ser Cys Thr Cys
        435                 440                 445
Pro Pro Gly Tyr Thr Gly Arg Asn Cys Ser Ala Pro Val Ser Arg Cys
    450                 455                 460
Glu His Ala Pro Cys His Asn Gly Ala Thr Cys His Glu Arg Gly His
465                 470                 475                 480
Arg Tyr Val Cys Glu Cys Ala Arg Gly Tyr Gly Gly Pro Asn Cys Gln
                485                 490                 495
Phe Leu Leu Pro Glu Leu Pro Pro Gly Pro Ala Val Val Asp Leu Thr
            500                 505                 510
Glu Lys Leu Glu Gly Gln Gly Gly
        515                 520
```

INFORMATION FOR SEQ ID NO : 4

LENGTH : 702

TYPE : amino acid

TOPOLOGY : linear

MOLECULE TYPE : protein

ORIGINAL SOURCE

ORGANISM : human

SEQUENCE DESCRIPTION : SEQ ID NO : 4 :

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Ser Gly Val Phe Glu Leu Lys Leu Gln Glu Phe Val Asn Lys Lys Gly
 1               5                  10                  15
Leu Leu Gly Asn Arg Asn Cys Cys Arg Gly Gly Ala Gly Pro Pro Pro
            20                  25                  30
Cys Ala Cys Arg Thr Phe Phe Arg Val Cys Leu Lys His Tyr Gln Ala
        35                  40                  45
Ser Val Ser Pro Glu Pro Pro Cys Thr Tyr Gly Ser Ala Val Thr Pro
    50                  55                  60
Val Leu Gly Val Asp Ser Phe Ser Leu Pro Asp Gly Gly Gly Ala Asp
65                  70                  75                  80
Ser Ala Phe Ser Asn Pro Ile Arg Phe Pro Phe Gly Phe Thr Trp Pro
                85                  90                  95
Gly Thr Phe Ser Leu Ile Ile Glu Ala Leu His Thr Asp Ser Pro Asp
            100                 105                 110
Asp Leu Ala Thr Glu Asn Pro Glu Arg Leu Ile Ser Arg Leu Ala Thr
        115                 120                 125
Gln Arg His Leu Thr Val Gly Glu Glu Trp Ser Gln Asp Leu His Ser
    130                 135                 140
Ser Gly Arg Thr Asp Leu Lys Tyr Ser Tyr Arg Phe Val Cys Asp Glu
145                 150                 155                 160
His Tyr Tyr Gly Glu Gly Cys Ser Val Phe Cys Arg Pro Arg Asp Asp
                165                 170                 175
Ala Phe Gly His Phe Thr Cys Gly Glu Arg Gly Glu Lys Val Cys Asn
            180                 185                 190
Pro Gly Trp Lys Gly Pro Tyr Cys Thr Glu Pro Ile Cys Leu Pro Gly
        195                 200                 205
Cys Asp Glu Gln His Gly Phe Cys Asp Lys Pro Gly Glu Cys Lys Cys
    210                 215                 220
```

Arg Val Gly Trp Gln Gly Arg Tyr Cys Asp Glu Cys Ile Arg Tyr Pro
225 230 235 240
Gly Cys Leu His Gly Thr Cys Gln Gln Pro Trp Gln Cys Asn Cys Gln
245 250 255
Glu Gly Trp Gly Gly Leu Phe Cys Asn Gln Asp Leu Asn Tyr Cys Thr
260 265 270
His His Lys Pro Cys Lys Asn Gly Ala Thr Cys Thr Asn Thr Gly Gln
275 280 285
Gly Ser Tyr Thr Cys Ser Cys Arg Pro Gly Tyr Thr Gly Ala Thr Cys
290 295 300
Glu Leu Gly Ile Asp Glu Cys Asp Pro Ser Pro Cys Lys Asn Gly Gly
305 310 315 320
Ser Cys Thr Asp Leu Glu Asn Ser Tyr Ser Cys Thr Cys Pro Pro Gly
325 330 335
Phe Tyr Gly Lys Ile Cys Glu Leu Ser Ala Met Thr Cys Ala Asp Gly
340 345 350
Pro Cys Phe Asn Gly Gly Arg Cys Ser Asp Ser Pro Asp Gly Gly Tyr
355 360 365
Ser Cys Arg Cys Pro Val Gly Tyr Ser Gly Phe Asn Cys Glu Lys Lys
370 375 380
Ile Asp Tyr Cys Ser Ser Ser Pro Cys Ser Asn Gly Ala Lys Cys Val
385 390 395 400
Asp Leu Gly Asp Ala Tyr Leu Cys Arg Cys Gln Ala Gly Phe Ser Gly
405 410 415
Arg His Cys Asp Asp Asn Val Asp Asp Cys Ala Ser Ser Pro Cys Ala
420 425 430
Asn Gly Gly Thr Cys Arg Asp Gly Val Asn Asp Phe Ser Cys Thr Cys
435 440 445
Pro Pro Gly Tyr Thr Gly Arg Asn Cys Ser Ala Pro Val Ser Arg Cys
450 455 460
Glu His Ala Pro Cys His Asn Gly Ala Thr Cys His Glu Arg Gly His
465 470 475 480

Arg Tyr Val Cys Glu Cys Ala Arg Gly Tyr Gly Gly Pro Asn Cys Gln
485 490 495
Phe Leu Leu Pro Glu Leu Pro Pro Gly Pro Ala Val Val Asp Leu Thr
500 505 510
Glu Lys Leu Glu Gly Gln Gly Gly Pro Phe Pro Trp Val Ala Val Cys
515 520 525
Ala Gly Val Ile Leu Val Leu Met Leu Leu Leu Gly Cys Ala Ala Val
530 535 540
Val Val Cys Val Arg Leu Arg Leu Gln Lys His Arg Pro Pro Ala Asp
545 550 555 560
Pro Cys Arg Gly Glu Thr Glu Thr Met Asn Asn Leu Ala Asn Cys Gln
565 570 575
Arg Glu Lys Asp Ile Ser Val Ser Ile Ile Gly Ala Thr Gln Ile Lys
580 585 590
Asn Thr Asn Lys Lys Ala Asp Phe His Gly Asp His Ser Ala Asp Lys
595 600 605
Asn Gly Phe Lys Ala Arg Tyr Pro Ala Val Asp Tyr Asn Leu Val Gln
610 615 620
Asp Leu Lys Gly Asp Asp Thr Ala Val Arg Asp Ala His Ser Lys Arg
625 630 635 640
Asp Thr Lys Cys Gln Pro Gln Gly Ser Ser Gly Glu Glu Lys Gly Thr
645 650 655
Pro Thr Thr Leu Arg Gly Gly Glu Ala Ser Glu Arg Lys Arg Pro Asp
660 665 670
Ser Gly Cys Ser Thr Ser Lys Asp Thr Lys Tyr Gln Ser Val Tyr Val
675 680 685
Ile Ser Glu Glu Lys Asp Glu Cys Val Ile Ala Thr Glu Val
690 695 700

INFORMATION FOR SEQ ID NO : 5

LENGTH : 198

TYPE : amino acid

TOPOLOGY : linear

MOLECULE TYPE : protein

ORIGINAL SOURCE

ORGANISM : human

SEQUENCE DESCRIPTION : SEQ ID NO : 5 :

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Ser Gly Gln Phe Glu Leu Glu Ile Leu Ser Met Gln Asn Val Asn Gly
 1               5                  10                  15
Glu Leu Gln Asn Gly Asn Cys Cys Gly Gly Ala Arg Asn Pro Gly Asp
            20                  25                  30
Arg Lys Cys Thr Arg Asp Glu Cys Asp Thr Tyr Phe Lys Val Cys Leu
        35                  40                  45
Lys Glu Tyr Gln Ser Arg Val Thr Ala Gly Gly Pro Cys Ser Phe Gly
    50                  55                  60
Ser Gly Ser Thr Pro Val Ile Gly Gly Asn Thr Phe Asn Leu Lys Ala
65                  70                  75                  80
Ser Arg Gly Asn Asp Arg Asn Arg Ile Val Leu Pro Phe Ser Phe Ala
                85                  90                  95
Trp Pro Arg Ser Tyr Thr Leu Leu Val Glu Ala Trp Asp Ser Ser Asn
            100                 105                 110
Asp Thr Val Gln Pro Asp Ser Ile Ile Glu Lys Ala Ser His Ser Gly
        115                 120                 125
Met Ile Asn Pro Ser Arg Gln Trp Gln Thr Leu Lys Gln Asn Thr Gly
    130                 135                 140
Val Ala His Phe Glu Tyr Gln Ile Arg Val Thr Cys Asp Asp Tyr Tyr
145                 150                 155                 160
Tyr Gly Phe Gly Cys Asn Lys Phe Cys Arg Pro Arg Asp Asp Phe Phe
                165                 170                 175
Gly His Tyr Ala Cys Asp Gln Asn Gly Asn Lys Thr Cys Met Glu Gly
            180                 185                 190
Trp Met Gly Pro Glu Cys
        195             198
```

INFORMATION FOR SEQ ID NO : 6

LENGTH : 1036

TYPE : amino acid

TOPOLOGY : linear

MOLECULE TYPE : protein

ORIGINAL SOURCE

ORGANISM : human

SEQUENCE DESCRIPTION : SEQ ID NO : 6 :

Ser Gly Gln Phe Glu Leu Glu Ile Leu Ser Met Gln Asn Val Asn Gly
1 5 10 15

Glu Leu Gln Asn Gly Asn Cys Cys Gly Gly Ala Arg Asn Pro Gly Asp
20 25 30

Arg Lys Cys Thr Arg Asp Glu Cys Asp Thr Tyr Phe Lys Val Cys Leu
35 40 45

Lys Glu Tyr Gln Ser Arg Val Thr Ala Gly Gly Pro Cys Ser Phe Gly
50 55 60

Ser Gly Ser Thr Pro Val Ile Gly Gly Asn Thr Phe Asn Leu Lys Ala
65 70 75 80

Ser Arg Gly Asn Asp Arg Asn Arg Ile Val Leu Pro Phe Ser Phe Ala
85 90 95

Trp Pro Arg Ser Tyr Thr Leu Leu Val Glu Ala Trp Asp Ser Ser Asn
100 105 110

Asp Thr Val Gln Pro Asp Ser Ile Ile Glu Lys Ala Ser His Ser Gly
115 120 125

Met Ile Asn Pro Ser Arg Gln Trp Gln Thr Leu Lys Gln Asn Thr Gly
130 135 140

Val Ala His Phe Glu Tyr Gln Ile Arg Val Thr Cys Asp Asp Tyr Tyr
145 150 155 160

Tyr Gly Phe Gly Cys Asn Lys Phe Cys Arg Pro Arg Asp Asp Phe Phe
165 170 175

Gly His Tyr Ala Cys Asp Gln Asn Gly Asn Lys Thr Cys Met Glu Gly
180 185 190

Trp Met Gly Pro Glu Cys Asn Arg Ala Ile Cys Arg Gln Gly Cys Ser
195 200 205
Pro Lys His Gly Ser Cys Lys Leu Pro Gly Asp Cys Arg Cys Gln Tyr
210 215 220
Gly Trp Gln Gly Leu Tyr Cys Asp Lys Cys Ile Pro His Pro Gly Cys
225 230 235 240
Val His Gly Ile Cys Asn Glu Pro Trp Gln Cys Leu Cys Glu Thr Asn
245 250 255
Trp Gly Gly Gln Leu Cys Asp Lys Asp Leu Asn Tyr Cys Gly Thr His
260 265 270
Gln Pro Cys Leu Asn Gly Gly Thr Cys Ser Asn Thr Gly Pro Asp Lys
275 280 285
Tyr Gln Cys Ser Cys Pro Glu Gly Tyr Ser Gly Pro Asn Cys Glu Ile
290 295 300
Ala Glu His Ala Cys Leu Ser Asp Pro Cys His Asn Arg Gly Ser Cys
305 310 315 320
Lys Glu Thr Ser Leu Gly Phe Glu Cys Glu Cys Ser Pro Gly Trp Thr
325 330 335
Gly Pro Thr Cys Ser Thr Asn Ile Asp Asp Cys Ser Pro Asn Asn Cys
340 345 350
Ser His Gly Gly Thr Cys Gln Asp Leu Val Asn Gly Phe Lys Cys Val
355 360 365
Cys Pro Pro Gln Trp Thr Gly Lys Thr Cys Gln Leu Asp Ala Asn Glu
370 375 380
Cys Glu Ala Lys Pro Cys Val Asn Ala Lys Ser Cys Lys Asn Leu Ile
385 390 395 400
Ala Ser Tyr Tyr Cys Asp Cys Leu Pro Gly Trp Met Gly Gln Asn Cys
405 410 415
Asp Ile Asn Ile Asn Asp Cys Leu Gly Gln Cys Gln Asn Asp Ala Ser
420 425 430
Cys Arg Asp Leu Val Asn Gly Tyr Arg Cys Ile Cys Pro Pro Gly Tyr
435 440 445

Ala Gly Asp His Cys Glu Arg Asp Ile Asp Glu Cys Ala Ser Asn Pro
450 455 460
Cys Leu Asn Gly Gly His Cys Gln Asn Glu Ile Asn Arg Phe Gln Cys
465 470 475 480
Leu Cys Pro Thr Gly Phe Ser Gly Asn Leu Cys Gln Leu Asp Ile Asp
485 490 495
Tyr Cys Glu Pro Asn Pro Cys Gln Asn Gly Ala Gln Cys Tyr Asn Arg
500 505 510
Ala Ser Asp Tyr Phe Cys Lys Cys Pro Glu Asp Tyr Glu Gly Lys Asn
515 520 525
Cys Ser His Leu Lys Asp His Cys Arg Thr Thr Pro Cys Glu Val Ile
530 535 540
Asp Ser Cys Thr Val Ala Met Ala Ser Asn Asp Thr Pro Glu Gly Val
545 550 555 560
Arg Tyr Ile Ser Ser Asn Val Cys Gly Pro His Gly Lys Cys Lys Ser
565 570 575
Gln Ser Gly Gly Lys Phe Thr Cys Asp Cys Asn Lys Gly Phe Thr Gly
580 585 590
Thr Tyr Cys His Glu Asn Ile Asn Asp Cys Glu Ser Asn Pro Cys Arg
595 600 605
Asn Gly Gly Thr Cys Ile Asp Gly Val Asn Ser Tyr Lys Cys Ile Cys
610 615 620
Ser Asp Gly Trp Glu Gly Ala Tyr Cys Glu Thr Asn Ile Asn Asp Cys
625 630 635 640
Ser Gln Asn Pro Cys His Asn Gly Gly Thr Cys Arg Asp Leu Val Asn
645 650 655
Asp Phe Tyr Cys Asp Cys Lys Asn Gly Trp Lys Gly Lys Thr Cys His
660 665 670
Ser Arg Asp Ser Gln Cys Asp Glu Ala Thr Cys Asn Asn Gly Gly Thr
675 680 685
Cys Tyr Asp Glu Gly Asp Ala Phe Lys Cys Met Cys Pro Gly Gly Trp
690 695 700

Glu Gly Thr Thr Cys Asn Ile Ala Arg Asn Ser Ser Cys Leu Pro Asn
705 710 715 720

Pro Cys His Asn Gly Gly Thr Cys Val Val Asn Gly Glu Ser Phe Thr
725 730 735

Cys Val Cys Lys Glu Gly Trp Glu Gly Pro Ile Cys Ala Gln Asn Thr
740 745 750

Asn Asp Cys Ser Pro His Pro Cys Tyr Asn Ser Gly Thr Cys Val Asp
755 760 765

Gly Asp Asn Trp Tyr Arg Cys Glu Cys Ala Pro Gly Phe Ala Gly Pro
770 775 780

Asp Cys Arg Ile Asn Ile Asn Glu Cys Gln Ser Ser Pro Cys Ala Phe
785 790 795 800

Gly Ala Thr Cys Val Asp Glu Ile Asn Gly Tyr Arg Cys Val Cys Pro
805 810 815

Pro Gly His Ser Gly Ala Lys Cys Gln Glu Val Ser Gly Arg Pro Cys
820 825 830

Ile Thr Met Gly Ser Val Ile Pro Asp Gly Ala Lys Trp Asp Asp Asp
835 840 845

Cys Asn Thr Cys Gln Cys Leu Asn Gly Arg Ile Ala Cys Ser Lys Val
850 855 860

Trp Cys Gly Pro Arg Pro Cys Leu Leu His Lys Gly His Ser Glu Cys
865 870 875 880

Pro Ser Gly Gln Ser Cys Ile Pro Ile Leu Asp Asp Gln Cys Phe Val
885 890 895

His Pro Cys Thr Gly Val Gly Glu Cys Arg Ser Ser Ser Leu Gln Pro
900 905 910

Val Lys Thr Lys Cys Thr Ser Asp Ser Tyr Tyr Gln Asp Asn Cys Ala
915 920 925

Asn Ile Thr Phe Thr Phe Asn Lys Glu Met Met Ser Pro Gly Leu Thr
930 935 940

Thr Glu His Ile Cys Ser Glu Leu Arg Asn Leu Asn Ile Leu Lys Asn
945 950 955 960

Val Ser Ala Glu Tyr Ser Ile Tyr Ile Ala Cys Glu Pro Ser Pro Ser
965 970 975
Ala Asn Asn Glu Ile His Val Ala Ile Ser Ala Glu Asp Ile Arg Asp
980 985 990
Asp Gly Asn Pro Ile Lys Glu Ile Thr Asp Lys Ile Ile Asp Leu Val
995 1000 1005
Ser Lys Arg Asp Gly Asn Ser Ser Leu Ile Ala Ala Val Ala Glu Val
1010 1015 1020
Arg Val Gln Arg Arg Pro Leu Lys Asn Arg Thr Asp
1025 1030 1035

INFORMATION FOR SEQ ID NO : 7
LENGTH : 1187
TYPE : amino acid
TOPOLOGY : linear
MOLECULE TYPE : protein
ORIGINAL SOURCE
ORGANISM : human
SEQUENCE DESCRIPTION : SEQ ID NO : 7 :
Ser Gly Gln Phe Glu Leu Glu Ile Leu Ser Met Gln Asn Val Asn Gly
1 5 10 15
Glu Leu Gln Asn Gly Asn Cys Cys Gly Gly Ala Arg Asn Pro Gly Asp
20 25 30
Arg Lys Cys Thr Arg Asp Glu Cys Asp Thr Tyr Phe Lys Val Cys Leu
35 40 45
Lys Glu Tyr Gln Ser Arg Val Thr Ala Gly Gly Pro Cys Ser Phe Gly
50 55 60
Ser Gly Ser Thr Pro Val Ile Gly Gly Asn Thr Phe Asn Leu Lys Ala
65 70 75 80
Ser Arg Gly Asn Asp Arg Asn Arg Ile Val Leu Pro Phe Ser Phe Ala
85 90 95
Trp Pro Arg Ser Tyr Thr Leu Leu Val Glu Ala Trp Asp Ser Ser Asn

100 105 110

Asp Thr Val Gln Pro Asp Ser Ile Ile Glu Lys Ala Ser His Ser Gly

115 120 125

Met Ile Asn Pro Ser Arg Gln Trp Gln Thr Leu Lys Gln Asn Thr Gly

130 135 140

Val Ala His Phe Glu Tyr Gln Ile Arg Val Thr Cys Asp Asp Tyr Tyr

145 150 155 160

Tyr Gly Phe Gly Cys Asn Lys Phe Cys Arg Pro Arg Asp Asp Phe Phe

165 170 175

Gly His Tyr Ala Cys Asp Gln Asn Gly Asn Lys Thr Cys Met Glu Gly

180 185 190

Trp Met Gly Pro Glu Cys Asn Arg Ala Ile Cys Arg Gln Gly Cys Ser

195 200 205

Pro Lys His Gly Ser Cys Lys Leu Pro Gly Asp Cys Arg Cys Gln Tyr

210 215 220

Gly Trp Gln Gly Leu Tyr Cys Asp Lys Cys Ile Pro His Pro Gly Cys

225 230 235 240

Val His Gly Ile Cys Asn Glu Pro Trp Gln Cys Leu Cys Glu Thr Asn

245 250 255

Trp Gly Gly Gln Leu Cys Asp Lys Asp Leu Asn Tyr Cys Gly Thr His

260 265 270

Gln Pro Cys Leu Asn Gly Gly Thr Cys Ser Asn Thr Gly Pro Asp Lys

275 280 285

Tyr Gln Cys Ser Cys Pro Glu Gly Tyr Ser Gly Pro Asn Cys Glu Ile

290 295 300

Ala Glu His Ala Cys Leu Ser Asp Pro Cys His Asn Arg Gly Ser Cys

305 310 315 320

Lys Glu Thr Ser Leu Gly Phe Glu Cys Glu Cys Ser Pro Gly Trp Thr

325 330 335

Gly Pro Thr Cys Ser Thr Asn Ile Asp Asp Cys Ser Pro Asn Asn Cys

340 345 350

Ser His Gly Gly Thr Cys Gln Asp Leu Val Asn Gly Phe Lys Cys Val

355 360 365

Cys Pro Pro Gln Trp Thr Gly Lys Thr Cys Gln Leu Asp Ala Asn Glu

370 375 380

Cys Glu Ala Lys Pro Cys Val Asn Ala Lys Ser Cys Lys Asn Leu Ile

385 390 395 400

Ala Ser Tyr Tyr Cys Asp Cys Leu Pro Gly Trp Met Gly Gln Asn Cys

405 410 415

Asp Ile Asn Ile Asn Asp Cys Leu Gly Gln Cys Gln Asn Asp Ala Ser

420 425 430

Cys Arg Asp Leu Val Asn Gly Tyr Arg Cys Ile Cys Pro Pro Gly Tyr

435 440 445

Ala Gly Asp His Cys Glu Arg Asp Ile Asp Glu Cys Ala Ser Asn Pro

450 455 460

Cys Leu Asn Gly Gly His Cys Gln Asn Glu Ile Asn Arg Phe Gln Cys

465 470 475 480

Leu Cys Pro Thr Gly Phe Ser Gly Asn Leu Cys Gln Leu Asp Ile Asp

485 490 495

Tyr Cys Glu Pro Asn Pro Cys Gln Asn Gly Ala Gln Cys Tyr Asn Arg

500 505 510

Ala Ser Asp Tyr Phe Cys Lys Cys Pro Glu Asp Tyr Glu Gly Lys Asn

515 520 525

Cys Ser His Leu Lys Asp His Cys Arg Thr Thr Pro Cys Glu Val Ile

530 535 540

Asp Ser Cys Thr Val Ala Met Ala Ser Asn Asp Thr Pro Glu Gly Val

545 550 555 560

Arg Tyr Ile Ser Ser Asn Val Cys Gly Pro His Gly Lys Cys Lys Ser

565 570 575

Gln Ser Gly Gly Lys Phe Thr Cys Asp Cys Asn Lys Gly Phe Thr Gly

580 585 590

Thr Tyr Cys His Glu Asn Ile Asn Asp Cys Glu Ser Asn Pro Cys Arg

595 600 605

Asn Gly Gly Thr Cys Ile Asp Gly Val Asn Ser Tyr Lys Cys Ile Cys

610 615 620

Ser Asp Gly Trp Glu Gly Ala Tyr Cys Glu Thr Asn Ile Asn Asp Cys

625 630 635 640

Ser Gln Asn Pro Cys His Asn Gly Gly Thr Cys Arg Asp Leu Val Asn

645 650 655

Asp Phe Tyr Cys Asp Cys Lys Asn Gly Trp Lys Gly Lys Thr Cys His

660 665 670

Ser Arg Asp Ser Gln Cys Asp Glu Ala Thr Cys Asn Asn Gly Gly Thr

675 680 685

Cys Tyr Asp Glu Gly Asp Ala Phe Lys Cys Met Cys Pro Gly Gly Trp

690 695 700

Glu Gly Thr Thr Cys Asn Ile Ala Arg Asn Ser Ser Cys Leu Pro Asn

705 710 715 720

Pro Cys His Asn Gly Gly Thr Cys Val Val Asn Gly Glu Ser Phe Thr

725 730 735

Cys Val Cys Lys Glu Gly Trp Glu Gly Pro Ile Cys Ala Gln Asn Thr

740 745 750

Asn Asp Cys Ser Pro His Pro Cys Tyr Asn Ser Gly Thr Cys Val Asp

755 760 765

Gly Asp Asn Trp Tyr Arg Cys Glu Cys Ala Pro Gly Phe Ala Gly Pro

770 775 780

Asp Cys Arg Ile Asn Ile Asn Glu Cys Gln Ser Ser Pro Cys Ala Phe

785 790 795 800

Gly Ala Thr Cys Val Asp Glu Ile Asn Gly Tyr Arg Cys Val Cys Pro

805 810 815

Pro Gly His Ser Gly Ala Lys Cys Gln Glu Val Ser Gly Arg Pro Cys

820 825 830

Ile Thr Met Gly Ser Val Ile Pro Asp Gly Ala Lys Trp Asp Asp Asp

835 840 845

Cys Asn Thr Cys Gln Cys Leu Asn Gly Arg Ile Ala Cys Ser Lys Val

850 855 860

Trp Cys Gly Pro Arg Pro Cys Leu Leu His Lys Gly His Ser Glu Cys

865 870 875 880
Pro Ser Gly Gln Ser Cys Ile Pro Ile Leu Asp Asp Gln Cys Phe Val
885 890 895
His Pro Cys Thr Gly Val Gly Glu Cys Arg Ser Ser Ser Leu Gln Pro
900 905 910
Val Lys Thr Lys Cys Thr Ser Asp Ser Tyr Tyr Gln Asp Asn Cys Ala
915 920 925
Asn Ile Thr Phe Thr Phe Asn Lys Glu Met Met Ser Pro Gly Leu Thr
930 935 940
Thr Glu His Ile Cys Ser Glu Leu Arg Asn Leu Asn Ile Leu Lys Asn
945 950 955 960
Val Ser Ala Glu Tyr Ser Ile Tyr Ile Ala Cys Glu Pro Ser Pro Ser
965 970 975
Ala Asn Asn Glu Ile His Val Ala Ile Ser Ala Glu Asp Ile Arg Asp
980 985 990
Asp Gly Asn Pro Ile Lys Glu Ile Thr Asp Lys Ile Ile Asp Leu Val
995 1000 1005
Ser Lys Arg Asp Gly Asn Ser Ser Leu Ile Ala Ala Val Ala Glu Val
1010 1015 1020
Arg Val Gln Arg Arg Pro Leu Lys Asn Arg Thr Asp Phe Leu Val Pro
1025 1030 1035 1040
Leu Leu Ser Ser Val Leu Thr Val Ala Trp Ile Cys Cys Leu Val Thr
1045 1050 1055
Ala Phe Tyr Trp Cys Leu Arg Lys Arg Arg Lys Pro Gly Ser His Thr
1060 1065 1070
His Ser Ala Ser Glu Asp Asn Thr Thr Asn Asn Val Arg Glu Gln Leu
1075 1080 1085
Asn Gln Ile Lys Asn Pro Ile Glu Lys His Gly Ala Asn Thr Val Pro
1090 1095 1100
Ile Lys Asp Tyr Glu Asn Lys Asn Ser Lys Met Ser Lys Ile Arg Thr
1105 1110 1115 1120
His Asn Ser Glu Val Glu Glu Asp Asp Met Asp Lys His Gln Gln Lys

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                    1125                1130                1135
Ala Arg Phe Ala Lys Gln Pro Ala Tyr Thr Leu Val Asp Arg Glu Glu
                1140                1145                1150
Lys Pro Pro Asn Gly Thr Pro Thr Lys His Pro Asn Trp Thr Asn Lys
        1155                1160                1165
Gln Asp Asn Arg Asp Leu Glu Ser Ala Gln Ser Leu Asn Arg Met Glu
    1170                1175                1180
Tyr Ile Val
1185    1187
```

INFORMATION FOR SEQ ID NO : 8

LENGTH : 2663 and 723

TYPE : nucleic acid and amino acid

STRANDEDNESS : double stranded and single stranded

TOPOLOGY : linear

MOLECULE TYPE : cDNA to mRNA, and amino acid

ORIGINAL SOURCE

ORGANISM : human

SEQUENCE DESCRIPTION : SEQ ID NO : 8 :

```
  CTTGGGAA GAGGCGGAGA CCGGCTTTTA AAGAAAGAAG TCCTGGGTCC TGCGGTCTGG   58
GGCGAGGCAA GGGCGCTTTT CTGCCCACGC TCCCCGTGGC CCATCGATCC CCCGCGCGTC  118
CGCCGCTGTT CTAAGGAGAG AAGTGGGGGC CCCCCAGGCT CGCGCGTGGA GCGAAGCAGC  178
ATG GGC AGT CGG TGC GCG CTG GCC CTG GCG GTG CTC TCG GCC TTG CTG     226
Met Gly Ser Arg Cys Ala Leu Ala Leu Ala Val Leu Ser Ala Leu Leu
    -20                 -15                 -10
TGT CAG GTC TGG AGC TCT GGG GTG TTC GAA CTG AAG CTG CAG GAG TTC     274
Cys Gln Val Trp Ser Ser Gly Val Phe Glu Leu Lys Leu Gln Glu Phe
 -5              -1  1                  5                  10
GTC AAC AAG AAG GGG CTG CTG GGG AAC CGC AAC TGC TGC CGC GGG GGC     322
Val Asn Lys Lys Gly Leu Leu Gly Asn Arg Asn Cys Cys Arg Gly Gly
                15                  20                  25
GCG GGG CCA CCG CCG TGC GCC TGC CGG ACC TTC TTC CGC GTG TGC CTC     370
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Ala Gly Pro Pro Pro Cys Ala Cys Arg Thr Phe Phe Arg Val Cys Leu
          30                  35                  40
AAG CAC TAC CAG GCC AGC GTG TCC CCC GAG CCG CCC TGC ACC TAC GGC    418
Lys His Tyr Gln Ala Ser Val Ser Pro Glu Pro Pro Cys Thr Tyr Gly
     45                  50                  55
AGC GCC GTC ACC CCC GTG CTG GGC GTC GAC TCC TTC AGT CTG CCC GAC    466
Ser Ala Val Thr Pro Val Leu Gly Val Asp Ser Phe Ser Leu Pro Asp
 60                  65                  70                  75
GGC GGG GGC GCC GAC TCC GCG TTC AGC AAC CCC ATC CGC TTC CCC TTC    514
Gly Gly Gly Ala Asp Ser Ala Phe Ser Asn Pro Ile Arg Phe Pro Phe
                 80                  85                  90
GGC TTC ACC TGG CCG GGC ACC TTC TCT CTG ATT ATT GAA GCT CTC CAC    562
Gly Phe Thr Trp Pro Gly Thr Phe Ser Leu Ile Ile Glu Ala Leu His
                 95                 100                 105
ACA GAT TCT CCT GAT GAC CTC GCA ACA GAA AAC CCA GAA AGA CTC ATC    610
Thr Asp Ser Pro Asp Asp Leu Ala Thr Glu Asn Pro Glu Arg Leu Ile
        110                 115                 120
AGC CGC CTG GCC ACC CAG AGG CAC CTG ACG GTG GGC GAG GAG TGG TCC    658
Ser Arg Leu Ala Thr Gln Arg His Leu Thr Val Gly Glu Glu Trp Ser
    125                 130                 135
CAG GAC CTG CAC AGC AGC GGC CGC ACG GAC CTC AAG TAC TCC TAC CGC    706
Gln Asp Leu His Ser Ser Gly Arg Thr Asp Leu Lys Tyr Ser Tyr Arg
140                 145                 150                 155
TTC GTG TGT GAC GAA CAC TAC TAC GGA GAG GGC TGC TCC GTT TTC TGC    754
Phe Val Cys Asp Glu His Tyr Tyr Gly Glu Gly Cys Ser Val Phe Cys
                160                 165                 170
CGT CCC CGG GAC GAT GCC TTC GGC CAC TTC ACC TGT GGG GAG CGT GGG    802
Arg Pro Arg Asp Asp Ala Phe Gly His Phe Thr Cys Gly Glu Arg Gly
                175                 180                 185
GAG AAA GTG TGC AAC CCT GGC TGG AAA GGG CCC TAC TGC ACA GAG CCG    850
Glu Lys Val Cys Asn Pro Gly Trp Lys Gly Pro Tyr Cys Thr Glu Pro
        190                 195                 200
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ATC TGC CTG CCT GGA TGT GAT GAG CAG CAT GGA TTT TGT GAC AAA CCA     898
Ile Cys Leu Pro Gly Cys Asp Glu Gln His Gly Phe Cys Asp Lys Pro
    205                 210                 215
GGG GAA TGC AAG TGC AGA GTG GGC TGG CAG GGC CGG TAC TGT GAC GAG     946
Gly Glu Cys Lys Cys Arg Val Gly Trp Gln Gly Arg Tyr Cys Asp Glu
220                 225                 230                 235
TGT ATC CGC TAT CCA GGC TGT CTC CAT GGC ACC TGC CAG CAG CCC TGG     994
Cys Ile Arg Tyr Pro Gly Cys Leu His Gly Thr Cys Gln Gln Pro Trp
                240                 245                 250
CAG TGC AAC TGC CAG GAA GGC TGG GGG GGC CTT TTC TGC AAC CAG GAC    1042
Gln Cys Asn Cys Gln Glu Gly Trp Gly Gly Leu Phe Cys Asn Gln Asp
            255                 260                 265
CTG AAC TAC TGC ACA CAC CAT AAG CCC TGC AAG AAT GGA GCC ACC TGC    1090
Leu Asn Tyr Cys Thr His His Lys Pro Cys Lys Asn Gly Ala Thr Cys
        270                 275                 280
ACC AAC ACG GGC CAG GGG AGC TAC ACT TGC TCT TGC CGG CCT GGG TAC    1138
Thr Asn Thr Gly Gln Gly Ser Tyr Thr Cys Ser Cys Arg Pro Gly Tyr
    285                 290                 295
ACA GGT GCC ACC TGC GAG CTG GGG ATT GAC GAG TGT GAC CCC AGC CCT    1186
Thr Gly Ala Thr Cys Glu Leu Gly Ile Asp Glu Cys Asp Pro Ser Pro
300                 305                 310                 315
TGT AAG AAC GGA GGG AGC TGC ACG GAT CTC GAG AAC AGC TAC TCC TGT    1234
Cys Lys Asn Gly Gly Ser Cys Thr Asp Leu Glu Asn Ser Tyr Ser Cys
                320                 325                 330
ACC TGC CCA CCC GGC TTC TAC GGC AAA ATC TGT GAA TTG AGT GCC ATG    1282
Thr Cys Pro Pro Gly Phe Tyr Gly Lys Ile Cys Glu Leu Ser Ala Met
            335                 340                 345
ACC TGT GCG GAC GGC CCT TGC TTT AAC GGG GGT CGG TGC TCA GAC AGC    1330
Thr Cys Ala Asp Gly Pro Cys Phe Asn Gly Gly Arg Cys Ser Asp Ser
        350                 355                 360
CCC GAT GGA GGG TAC AGC TGC CGC TGC CCC GTG GGC TAC TCC GGC TTC    1378
Pro Asp Gly Gly Tyr Ser Cys Arg Cys Pro Val Gly Tyr Ser Gly Phe
```

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                365                 370                 375
AAC TGT GAG AAG AAA ATT GAC TAC TGC AGC TCT TCA CCC TGT TCT AAT   1426
Asn Cys Glu Lys Lys Ile Asp Tyr Cys Ser Ser Ser Pro Cys Ser Asn
380                 385                 390                 395
GGT GCC AAG TGT GTG GAC CTC GGT GAT GCC TAC CTG TGC CGC TGC CAG   1474
Gly Ala Lys Cys Val Asp Leu Gly Asp Ala Tyr Leu Cys Arg Cys Gln
                    400                 405                 410
GCC GGC TTC TCG GGG AGG CAC TGT GAC GAC AAC GTG GAC GAC TGC GCC   1522
Ala Gly Phe Ser Gly Arg His Cys Asp Asp Asn Val Asp Asp Cys Ala
                415                 420                 425
TCC TCC CCG TGC GCC AAC GGG GGC ACC TGC CGG GAT GGC GTG AAC GAC   1570
Ser Ser Pro Cys Ala Asn Gly Gly Thr Cys Arg Asp Gly Val Asn Asp
        430                 435                 440
TTC TCC TGC ACC TGC CCG CCT GGC TAC ACG GGC AGG AAC TGC AGT GCC   1618
Phe Ser Cys Thr Cys Pro Pro Gly Tyr Thr Gly Arg Asn Cys Ser Ala
    445                 450                 455
CCC GTC AGC AGG TGC GAG CAC GCA CCC TGC CAC AAT GGG GCC ACC TGC   1666
Pro Val Ser Arg Cys Glu His Ala Pro Cys His Asn Gly Ala Thr Cys
460                 465                 470                 475
CAC GAG AGG GGC CAC CGC TAT GTG TGC GAG TGT GCC CGA GGC TAC GGG   1714
His Glu Arg Gly His Arg Tyr Val Cys Glu Cys Ala Arg Gly Tyr Gly
                    480                 485                 490
GGT CCC AAC TGC CAG TTC CTG CTC CCC GAG CTG CCC CCG GGC CCA GCG   1762
Gly Pro Asn Cys Gln Phe Leu Leu Pro Glu Leu Pro Pro Gly Pro Ala
                495                 500                 505
GTG GTG GAC CTC ACT GAG AAG CTA GAG GGC CAG GGC GGG CCA TTC CCC   1810
Val Val Asp Leu Thr Glu Lys Leu Glu Gly Gln Gly Gly Pro Phe Pro
        510                 515                 520
TGG GTG GCC GTG TGC GCC GGG GTC ATC CTT GTC CTC ATG CTG CTG CTG   1858
Trp Val Ala Val Cys Ala Gly Val Ile Leu Val Leu Met Leu Leu Leu
    525                 530                 535
GGC TGT GCC GCT GTG GTG GTC TGC GTC CGG CTG AGG CTG CAG AAG CAC   1906
```

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Gly Cys Ala Ala Val Val Val Cys Val Arg Leu Arg Leu Gln Lys His
540                 545                 550                 555
CGG CCC CCA GCC GAC CCC TGC CGG GGG GAG ACG GAG ACC ATG AAC AAC   1954
Arg Pro Pro Ala Asp Pro Cys Arg Gly Glu Thr Glu Thr Met Asn Asn
                560                 565                 570
CTG GCC AAC TGC CAG CGT GAG AAG GAC ATC TCA GTC AGC ATC ATC GGG   2002
Leu Ala Asn Cys Gln Arg Glu Lys Asp Ile Ser Val Ser Ile Ile Gly
            575                 580                 585
GCC ACG CAG ATC AAG AAC ACC AAC AAG AAG GCG GAC TTC CAC GGG GAC   2050
Ala Thr Gln Ile Lys Asn Thr Asn Lys Lys Ala Asp Phe His Gly Asp
        590                 595                 600
CAC AGC GCC GAC AAG AAT GGC TTC AAG GCC CGC TAC CCA GCG GTG GAC   2098
His Ser Ala Asp Lys Asn Gly Phe Lys Ala Arg Tyr Pro Ala Val Asp
    605                 610                 615
TAT AAC CTC GTG CAG GAC CTC AAG GGT GAC GAC ACC GCC GTC AGG GAC   2146
Tyr Asn Leu Val Gln Asp Leu Lys Gly Asp Asp Thr Ala Val Arg Asp
620                 625                 630                 635
GCG CAC AGC AAG CGT GAC ACC AAG TGC CAG CCC CAG GGC TCC TCA GGG   2194
Ala His Ser Lys Arg Asp Thr Lys Cys Gln Pro Gln Gly Ser Ser Gly
                640                 645                 650
GAG GAG AAG GGG ACC CCG ACC ACA CTC AGG GGT GGA GAA GCA TCT GAA   2242
Glu Glu Lys Gly Thr Pro Thr Thr Leu Arg Gly Gly Glu Ala Ser Glu
            655                 660                 665
AGA AAA AGG CCG GAC TCG GGC TGT TCA ACT TCA AAA GAC ACC AAG TAC   2290
Arg Lys Arg Pro Asp Ser Gly Cys Ser Thr Ser Lys Asp Thr Lys Tyr
        670                 675                 680
CAG TCG GTG TAC GTC ATA TCC GAG GAG AAG GAT GAG TGC GTC ATA GCA   2338
Gln Ser Val Tyr Val Ile Ser Glu Glu Lys Asp Glu Cys Val Ile Ala
    685                 690                 695
ACT GAG GTG                                                      2347
Thr Glu Val
700
```

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TAAAATGGAA GTGAGATGGC AAGACTCCCG TTTCTCTTAA AATAAGTAAA ATTCCAAGGA 2407
TATATGCCCC AACGAATGCT GCTGAAGAGG AGGGAGGCCT CGTGGACTGC TGCTGAGAAA 2467
CCGAGTTCAG ACCGAGCAGG TTCTCCTCCT GAGGTCCTCG ACGCCTGCCG ACAGCCTGTC 2527
GCGGCCCGGC CGCCTGCGGC ACTGCCTTCC GTGACGTCGC CGTTGCACTA TGGACAGTTG 2587
CTCTTAAGAG AATATATATT TAAATGGGTG AACTGAATTA CGCATAAGAA GCATGCACTG 2647
CCTGAGTGTA TATTTT                                                  2663
```

INFORMATION FOR SEQ ID NO : 9

LENGTH : 4005 and 1218

TYPE : nucleic acid and amino acid

STRANDEDNESS : double stranded and single stranded

TOPOLOGY : linear and unknown

MOLECULE TYPE : cDNA to mRNA. and amino acid

ORIGINAL SOURCE

ORGANISM : human

SEQUENCE DESCRIPTION : SEQ ID NO : 9 :

```
                GGCCGGCC CGCGAGCTAG GCTGGTTTTT TTTTTTCTCC CCTCCCTCCC   48
CCCTTTTTCC ATGCAGCTGA TCTAAAAGGG AATAAAAGGC TGCGCATAAT CATAATAATA  108
AAAGAAGGGG AGCGCGAGAG AAGGAAAGAA AGCCGCGAGG TGGAAGAGGA GGGGGAGCGT  168
CTCAAAGAAG CGATCAGAAT AATAAAAGGA GGCCGGGCTC TTTGCCTTCT GGAACGGGCC  228
GCTCTTGAAA GGGCTTTTGA AAAGTGGTGT TGTTTTCCAG TCGTGCATGC TCCAATCGGC  288
GGAGTATATT AGAGCCGGGA CGCGGCGGCC GCAGGGGCAG CGGCGACGGC AGCACCGGCG  348
GCAGCACCAG CGCGAACAGC AGCGGCGGCG TCCCGAGTGC CCGCGGCGCG CGGCGCAGCG  408
    ATG CGT TCC CCA CGG ACG CGC GGC CGG TCC GGG CGC CCC CTA AGC      453
    Met Arg Ser Pro Arg Thr Arg Gly Arg Ser Gly Arg Pro Leu Ser
    -31 -30                 -25                 -20
CTC CTG CTC GCC CTG CTC TGT GCC CTG CGA GCC AAG GTG TGT GGG GCC     501
Leu Leu Leu Ala Leu Leu Cys Ala Leu Arg Ala Lys Val Cys Gly Ala
    -15                 -10                 -5                 -1
TCG GGT CAG TTC GAG TTG GAG ATC CTG TCC ATG CAG AAC GTG AAC GGG     549
Ser Gly Gln Phe Glu Leu Glu Ile Leu Ser Met Gln Asn Val Asn Gly
 1              5                  10                  15
```

```
GAG CTG CAG AAC GGG AAC TGC TGC GGC GGC GCC CGG AAC CCG GGA GAC     597
Glu Leu Gln Asn Gly Asn Cys Cys Gly Gly Ala Arg Asn Pro Gly Asp
                 20                  25                  30
CGC AAG TGC ACC CGC GAC GAG TGT GAC ACA TAC TTC AAA GTG TGC CTC     645
Arg Lys Cys Thr Arg Asp Glu Cys Asp Thr Tyr Phe Lys Val Cys Leu
             35                  40                  45
AAG GAG TAT CAG TCC CGC GTC ACG GCC GGG GGG CCC TGC AGC TTC GGC     693
Lys Glu Tyr Gln Ser Arg Val Thr Ala Gly Gly Pro Cys Ser Phe Gly
         50                  55                  60
TCA GGG TCC ACG CCT GTC ATC GGG GGC AAC ACC TTC AAC CTC AAG GCC     741
Ser Gly Ser Thr Pro Val Ile Gly Gly Asn Thr Phe Asn Leu Lys Ala
 65                  70                  75                  80
AGC CGC GGC AAC GAC CGC AAC CGC ATC GTG CTG CCT TTC AGT TTC GCC     789
Ser Arg Gly Asn Asp Arg Asn Arg Ile Val Leu Pro Phe Ser Phe Ala
                 85                  90                  95
TGG CCG AGG TCC TAT ACG TTG CTT GTG GAG GCG TGG GAT TCC AGT AAT     837
Trp Pro Arg Ser Tyr Thr Leu Leu Val Glu Ala Trp Asp Ser Ser Asn
             100                 105                 110
GAC ACC GTT CAA CCT GAC AGT ATT ATT GAA AAG GCT TCT CAC TCG GGC     885
Asp Thr Val Gln Pro Asp Ser Ile Ile Glu Lys Ala Ser His Ser Gly
         115                 120                 125
ATG ATC AAC CCC AGC CGG CAG TGG CAG ACG CTG AAG CAG AAC ACG GGC     933
Met Ile Asn Pro Ser Arg Gln Trp Gln Thr Leu Lys Gln Asn Thr Gly
     130                 135                 140
GTT GCC CAC TTT GAG TAT CAG ATC CGC GTG ACC TGT GAT GAC TAC TAC     981
Val Ala His Phe Glu Tyr Gln Ile Arg Val Thr Cys Asp Asp Tyr Tyr
 145                 150                 155                 160
TAT GGC TTT GGC TGC AAT AAG TTC TGC CGC CCC AGA GAT GAC TTC TTT    1029
Tyr Gly Phe Gly Cys Asn Lys Phe Cys Arg Pro Arg Asp Asp Phe Phe
                 165                 170                 175
GGA CAC TAT GCC TGT GAC CAG AAT GGC AAC AAA ACT TGC ATG GAA GGC    1077
Gly His Tyr Ala Cys Asp Gln Asn Gly Asn Lys Thr Cys Met Glu Gly
```

```
                180                 185                 190
TGG ATG GGC CCC GAA TGT AAC AGA GCT ATT TGC CGA CAA GGC TGC AGT   1125
Trp Met Gly Pro Glu Cys Asn Arg Ala Ile Cys Arg Gln Gly Cys Ser
        195                 200                 205
CCT AAG CAT GGG TCT TGC AAA CTC CCA GGT GAC TGC AGG TGC CAG TAC   1173
Pro Lys His Gly Ser Cys Lys Leu Pro Gly Asp Cys Arg Cys Gln Tyr
    210                 215                 220
GGC TGG CAA GGC CTG TAC TGT GAT AAG TGC ATC CCA CAC CCG GGA TGC   1221
Gly Trp Gln Gly Leu Tyr Cys Asp Lys Cys Ile Pro His Pro Gly Cys
225                 230                 235                 240
GTC CAC GGC ATC TGT AAT GAG CCC TGG CAG TGC CTC TGT GAG ACC AAC   1269
Val His Gly Ile Cys Asn Glu Pro Trp Gln Cys Leu Cys Glu Thr Asn
                245                 250                 255
TGG GGC GGC CAG CTC TGT GAC AAA GAT CTC AAT TAC TGT GGG ACT CAT   1317
Trp Gly Gly Gln Leu Cys Asp Lys Asp Leu Asn Tyr Cys Gly Thr His
            260                 265                 270
CAG CCG TGT CTC AAC GGG GGA ACT TGT AGC AAC ACA GGC CCT GAC AAA   1365
Gln Pro Cys Leu Asn Gly Gly Thr Cys Ser Asn Thr Gly Pro Asp Lys
        275                 280                 285
TAT CAG TGT TCC TGC CCT GAG GGG TAT TCA GGA CCC AAC TGT GAA ATT   1413
Tyr Gln Cys Ser Cys Pro Glu Gly Tyr Ser Gly Pro Asn Cys Glu Ile
    290                 295                 300
GCT GAG CAC GCC TGC CTC TCT GAT CCC TGT CAC AAC AGA GGC AGC TGT   1461
Ala Glu His Ala Cys Leu Ser Asp Pro Cys His Asn Arg Gly Ser Cys
305                 310                 315                 320
AAG GAG ACC TCC CTG GGC TTT GAG TGT GAG TGT TCC CCA GGC TGG ACC   1509
Lys Glu Thr Ser Leu Gly Phe Glu Cys Glu Cys Ser Pro Gly Trp Thr
                325                 330                 335
GGC CCC ACA TGC TCT ACA AAC ATT GAT GAC TGT TCT CCT AAT AAC TGT   1557
Gly Pro Thr Cys Ser Thr Asn Ile Asp Asp Cys Ser Pro Asn Asn Cys
            340                 345                 350
TCC CAC GGG GGC ACC TGC CAG GAC CTG GTT AAC GGA TTT AAG TGT GTG   1605
```

Ser His Gly Gly Thr Cys Gln Asp Leu Val Asn Gly Phe Lys Cys Val	
355 360 365	
TGC CCC CCA CAG TGG ACT GGG AAA ACG TGC CAG TTA GAT GCA AAT GAA	1653
Cys Pro Pro Gln Trp Thr Gly Lys Thr Cys Gln Leu Asp Ala Asn Glu	
370 375 380	
TGT GAG GCC AAA CCT TGT GTA AAC GCC AAA TCC TGT AAG AAT CTC ATT	1701
Cys Glu Ala Lys Pro Cys Val Asn Ala Lys Ser Cys Lys Asn Leu Ile	
385 390 395 400	
GCC AGC TAC TAC TGC GAC TGT CTT CCC GGC TGG ATG GGT CAG AAT TGT	1749
Ala Ser Tyr Tyr Cys Asp Cys Leu Pro Gly Trp Met Gly Gln Asn Cys	
405 410 415	
GAC ATA AAT ATT AAT GAC TGC CTT GGC CAG TGT CAG AAT GAC GCC TCC	1797
Asp Ile Asn Ile Asn Asp Cys Leu Gly Gln Cys Gln Asn Asp Ala Ser	
420 425 430	
TGT CGG GAT TTG GTT AAT GGT TAT CGC TGT ATC TGT CCA CCT GGC TAT	1845
Cys Arg Asp Leu Val Asn Gly Tyr Arg Cys Ile Cys Pro Pro Gly Tyr	
435 440 445	
GCA GGC GAT CAC TGT GAG AGA GAC ATC GAT GAA TGT GCC AGC AAC CCC	1893
Ala Gly Asp His Cys Glu Arg Asp Ile Asp Glu Cys Ala Ser Asn Pro	
450 455 460	
TGT TTG AAT GGG GGT CAC TGT CAG AAT GAA ATC AAC AGA TTC CAG TGT	1941
Cys Leu Asn Gly Gly His Cys Gln Asn Glu Ile Asn Arg Phe Gln Cys	
465 470 475 480	
CTG TGT CCC ACT GGT TTC TCT GGA AAC CTC TGT CAG CTG GAC ATC GAT	1989
Leu Cys Pro Thr Gly Phe Ser Gly Asn Leu Cys Gln Leu Asp Ile Asp	
485 490 495	
TAT TGT GAG CCT AAT CCC TGC CAG AAC GGT GCC CAG TGC TAC AAC CGT	2037
Tyr Cys Glu Pro Asn Pro Cys Gln Asn Gly Ala Gln Cys Tyr Asn Arg	
500 505 510	
GCC AGT GAC TAT TTC TGC AAG TGC CCC GAG GAC TAT GAG GGC AAG AAC	2085
Ala Ser Asp Tyr Phe Cys Lys Cys Pro Glu Asp Tyr Glu Gly Lys Asn	
515 520 525	

```
TGC TCA CAC CTG AAA GAC CAC TGC CGC ACG ACC CCC TGT GAA GTG ATT   2133
Cys Ser His Leu Lys Asp His Cys Arg Thr Thr Pro Cys Glu Val Ile
    530                 535                 540
GAC AGC TGC ACA GTG GCC ATG GCT TCC AAC GAC ACA CCT GAA GGG GTG   2181
Asp Ser Cys Thr Val Ala Met Ala Ser Asn Asp Thr Pro Glu Gly Val
545                 550                 555                 560
CGG TAT ATT TCC TCC AAC GTC TGT GGT CCT CAC GGG AAG TGC AAG AGT   2229
Arg Tyr Ile Ser Ser Asn Val Cys Gly Pro His Gly Lys Cys Lys Ser
                565                 570                 575
CAG TCG GGA GGC AAA TTC ACC TGT GAC TGT AAC AAA GGC TTC ACG GGA   2277
Gln Ser Gly Gly Lys Phe Thr Cys Asp Cys Asn Lys Gly Phe Thr Gly
            580                 585                 590
ACA TAC TGC CAT GAA AAT ATT AAT GAC TGT GAG AGC AAC CCT TGT AGA   2325
Thr Tyr Cys His Glu Asn Ile Asn Asp Cys Glu Ser Asn Pro Cys Arg
        595                 600                 605
AAC GGT GGC ACT TGC ATC GAT GGT GTC AAC TCC TAC AAG TGC ATC TGT   2373
Asn Gly Gly Thr Cys Ile Asp Gly Val Asn Ser Tyr Lys Cys Ile Cys
    610                 615                 620
AGT GAC GGC TGG GAG GGG GCC TAC TGT GAA ACC AAT ATT AAT GAC TGC   2421
Ser Asp Gly Trp Glu Gly Ala Tyr Cys Glu Thr Asn Ile Asn Asp Cys
625                 630                 635                 640
AGC CAG AAC CCC TGC CAC AAT GGG GGC ACG TGT CGC GAC CTG GTC AAT   2469
Ser Gln Asn Pro Cys His Asn Gly Gly Thr Cys Arg Asp Leu Val Asn
                645                 650                 655
GAC TTC TAC TGT GAC TGT AAA AAT GGG TGG AAA GGA AAG ACC TGC CAC   2517
Asp Phe Tyr Cys Asp Cys Lys Asn Gly Trp Lys Gly Lys Thr Cys His
            660                 665                 670
TCA CGT GAC AGT CAG TGT GAT GAG GCC ACG TGC AAC AAC GGT GGC ACC   2565
Ser Arg Asp Ser Gln Cys Asp Glu Ala Thr Cys Asn Asn Gly Gly Thr
        675                 680                 685
TGC TAT GAT GAG GGG GAT GCT TTT AAG TGC ATG TGT CCT GGC GGC TGG   2613
Cys Tyr Asp Glu Gly Asp Ala Phe Lys Cys Met Cys Pro Gly Gly Trp
```

```
     690                 695                 700
GAA GGA ACA ACC TGT AAC ATA GCC CGA AAC AGT AGC TGC CTG CCC AAC   2661
Glu Gly Thr Thr Cys Asn Ile Ala Arg Asn Ser Ser Cys Leu Pro Asn
705                 710                 715                 720
CCC TGC CAT AAT GGG GGC ACA TGT GTG GTC AAC GGC GAG TCC TTT ACG   2709
Pro Cys His Asn Gly Gly Thr Cys Val Val Asn Gly Glu Ser Phe Thr
                725                 730                 735
TGC GTC TGC AAG GAA GGC TGG GAG GGG CCC ATC TGT GCT CAG AAT ACC   2757
Cys Val Cys Lys Glu Gly Trp Glu Gly Pro Ile Cys Ala Gln Asn Thr
            740                 745                 750
AAT GAC TGC AGC CCT CAT CCC TGT TAC AAC AGC GGC ACC TGT GTG GAT   2805
Asn Asp Cys Ser Pro His Pro Cys Tyr Asn Ser Gly Thr Cys Val Asp
        755                 760                 765
GGA GAC AAC TGG TAC CGG TGC GAA TGT GCC CCG GGT TTT GCT GGG CCC   2853
Gly Asp Asn Trp Tyr Arg Cys Glu Cys Ala Pro Gly Phe Ala Gly Pro
    770                 775                 780
GAC TGC AGA ATA AAC ATC AAT GAA TGC CAG TCT TCA CCT TGT GCC TTT   2901
Asp Cys Arg Ile Asn Ile Asn Glu Cys Gln Ser Ser Pro Cys Ala Phe
785                 790                 795                 800
GGA GCG ACC TGT GTG GAT GAG ATC AAT GGC TAC CGG TGT GTC TGC CCT   2949
Gly Ala Thr Cys Val Asp Glu Ile Asn Gly Tyr Arg Cys Val Cys Pro
                805                 810                 815
CCA GGG CAC AGT GGT GCC AAG TGC CAG GAA GTT TCA GGG AGA CCT TGC   2997
Pro Gly His Ser Gly Ala Lys Cys Gln Glu Val Ser Gly Arg Pro Cys
            820                 825                 830
ATC ACC ATG GGG AGT GTG ATA CCA GAT GGG GCC AAA TGG GAT GAT GAC   3045
Ile Thr Met Gly Ser Val Ile Pro Asp Gly Ala Lys Trp Asp Asp Asp
        835                 840                 845
TGT AAT ACC TGC CAG TGC CTG AAT GGA CGG ATC GCC TGC TCA AAG GTC   3093
Cys Asn Thr Cys Gln Cys Leu Asn Gly Arg Ile Ala Cys Ser Lys Val
    850                 855                 860
TGG TGT GGC CCT CGA CCT TGC CTG CTC CAC AAA GGG CAC AGC GAG TGC   3141
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Trp Cys Gly Pro Arg Pro Cys Leu Leu His Lys Gly His Ser Glu Cys
865                 870                 875                 880
CCC AGC GGG CAG AGC TGC ATC CCC ATC CTG GAC GAC CAG TGC TTC GTC   3189
Pro Ser Gly Gln Ser Cys Ile Pro Ile Leu Asp Asp Gln Cys Phe Val
                885                 890                 895
CAC CCC TGC ACT GGT GTG GGC GAG TGT CGG TCT TCC AGT CTC CAG CCG   3237
His Pro Cys Thr Gly Val Gly Glu Cys Arg Ser Ser Ser Leu Gln Pro
            900                 905                 910
GTG AAG ACA AAG TGC ACC TCT GAC TCC TAT TAC CAG GAT AAC TGT GCG   3285
Val Lys Thr Lys Cys Thr Ser Asp Ser Tyr Tyr Gln Asp Asn Cys Ala
        915                 920                 925
AAC ATC ACA TTT ACC TTT AAC AAG GAG ATG ATG TCA CCA GGT CTT ACT   3333
Asn Ile Thr Phe Thr Phe Asn Lys Glu Met Met Ser Pro Gly Leu Thr
    930                 935                 940
ACG GAG CAC ATT TGC AGT GAA TTG AGG AAT TTG AAT ATT TTG AAG AAT   3381
Thr Glu His Ile Cys Ser Glu Leu Arg Asn Leu Asn Ile Leu Lys Asn
945                 950                 955                 960
GTT TCC GCT GAA TAT TCA ATC TAC ATC GCT TGC GAG CCT TCC CCT TCA   3429
Val Ser Ala Glu Tyr Ser Ile Tyr Ile Ala Cys Glu Pro Ser Pro Ser
                965                 970                 975
GCG AAC AAT GAA ATA CAT GTG GCC ATT TCT GCT GAA GAT ATA CGG GAT   3477
Ala Asn Asn Glu Ile His Val Ala Ile Ser Ala Glu Asp Ile Arg Asp
            980                 985                 990
GAT GGG AAC CCG ATC AAG GAA ATC ACT GAC AAA ATA ATC GAT CTT GTT   3525
Asp Gly Asn Pro Ile Lys Glu Ile Thr Asp Lys Ile Ile Asp Leu Val
        995                 1000                1005
AGT AAA CGT GAT GGA AAC AGC TCG CTG ATT GCT GCC GTT GCA GAA GTA   3573
Ser Lys Arg Asp Gly Asn Ser Ser Leu Ile Ala Ala Val Ala Glu Val
    1010                1015                1020
AGA GTT CAG AGG CGG CCT CTG AAG AAC AGA ACA GAT TTC CTT GTT CCC   3621
Arg Val Gln Arg Arg Pro Leu Lys Asn Arg Thr Asp Phe Leu Val Pro
1025                1030                1035                1040
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TTG CTG AGC TCT GTC TTA ACT GTG GCT TGG ATC TGT TGC TTG GTG ACG   3669
Leu Leu Ser Ser Val Leu Thr Val Ala Trp Ile Cys Cys Leu Val Thr
                1045                1050                1055
GCC TTC TAC TGG TGC CTG CGG AAG CGG CGG AAG CCG GGC AGC CAC ACA   3717
Ala Phe Tyr Trp Cys Leu Arg Lys Arg Arg Lys Pro Gly Ser His Thr
            1060                1065                1070
CAC TCA GCC TCT GAG GAC AAC ACC ACC AAC AAC GTG CGG GAG CAG CTG   3765
His Ser Ala Ser Glu Asp Asn Thr Thr Asn Asn Val Arg Glu Gln Leu
        1075                1080                1085
AAC CAG ATC AAA AAC CCC ATT GAG AAA CAT GGG GCC AAC ACG GTC CCC   3813
Asn Gln Ile Lys Asn Pro Ile Glu Lys His Gly Ala Asn Thr Val Pro
    1090                1095                1100
ATC AAG GAT TAT GAG AAC AAG AAC TCC AAA ATG TCT AAA ATA AGG ACA   3861
Ile Lys Asp Tyr Glu Asn Lys Asn Ser Lys Met Ser Lys Ile Arg Thr
1105                1110                1115                1120
CAC AAT TCT GAA GTA GAA GAG GAC GAC ATG GAC AAA CAC CAG CAG AAA   3909
His Asn Ser Glu Val Glu Glu Asp Asp Met Asp Lys His Gln Gln Lys
            1125                1130                1135
GCC CGG TTT GCC AAG CAG CCG GCG TAC ACG CTG GTA GAC AGA GAA GAG   3957
Ala Arg Phe Ala Lys Gln Pro Ala Tyr Thr Leu Val Asp Arg Glu Glu
        1140                1145                1150
AAG CCC CCC AAC GGC ACG CCG ACA AAA CAC CCA AAC TGG ACA AAC AAA   4005
Lys Pro Pro Asn Gly Thr Pro Thr Lys His Pro Asn Trp Thr Asn Lys
    1155                1160                1165
CAG GAC AAC AGA GAC TTG GAA AGT GCC CAG AGC TTA AAC CGA ATG GAG   4053
Gln Asp Asn Arg Asp Leu Glu Ser Ala Gln Ser Leu Asn Arg Met Glu
    1170                1175                1180
TAC ATC GTA                                                      4062
Tyr Ile Val
1185    1187
TAGCAGACCG CGGGCACTGC CGCCGCTAGG TAGAGTCTGA GGGCTTGTAG TTCTTTAAAC  4122
TGTCGTGTCA TACTCGAGTC TGAGGCCGTT GCTGACTTAG AATCCCTGTG TTAATTTAAG  4182
```

TTTTGACAAG CTGGCTTACA CTGGCA 4208

INFORMATION FOR SEQ ID NO : 10

LENGTH : 27 and 8

TYPE : nucleic acid and amino acid

STRANDEDNESS : single stranded

TOPOLOGY : linear

MOLECULE TYPE : DNA and amino acid

ORIGINAL SOURCE : chemical synthesis

SEQUENCE DESCRIPTION : SEQ ID NO : 10 :

GAT TAT AAA GAT GAT GAT GAT AAA TGA 27

Asp Tyr Lys Asp Asp Asp Asp Lys

1 5 8

INFORMATION FOR SEQ ID NO : 11

LENGTH : 20

TYPE : nucleic acid

STRANDEDNESS : single stranded

TOPOLOGY : linear

MOLECULE TYPE : DNA

ORIGINAL SOURCE : chemical synthesis

SEQUENCE DESCRIPTION : SEQ ID NO : 11 :

TGGCARTGYA AYTGYCARGA

INFORMATION FOR SEQ ID NO : 12

LENGTH : 20

TYPE : nucleic acid

STRANDEDNESS : single stranded

TOPOLOGY : linear

MOLECULE TYPE : DNA

ORIGINAL SOURCE : chemical synthesis

SEQUENCE DESCRIPTION : SEQ ID NO : 12 :

ATYTTYTTYT CRCARTTRAA

INFORMATION FOR SEQ ID NO : 13
LENGTH : 20
TYPE : nucleic acid
STRANDEDNESS : single stranded
TOPOLOGY : linear
MOLECULE TYPE : DNA
ORIGINAL SOURCE : chemical synthesis
SEQUENCE DESCRIPTION : SEQ ID NO : 13 :
TGCSTSTGYG ANACCAACTG

INFORMATION FOR SEQ ID NO : 14
LENGTH : 20
TYPE : nucleic acid
STRANDEDNESS : single stranded
TOPOLOGY : linear
MOLECULE TYPE : DNA
ORIGINAL SOURCE : chemical synthesis
SEQUENCE DESCRIPTION : SEQ ID NO : 14 :
TTTATKTCRC AWKTCKGWCC

INFORMATION FOR SEQ ID NO : 15
LENGTH : 25
TYPE : nucleic acid
STRANDEDNESS : single stranded
TOPOLOGY : linear
MOLECULE TYPE : DNA
ORIGINAL SOURCE : chemical synthesis
SEQUENCE DESCRIPTION : SEQ ID NO : 15 :
TCGCGCGTGG AGCGAAGCAG CATGG

INFORMATION FOR SEQ ID NO : 16

LENGTH : 25

TYPE : nucleic acid

STRANDEDNESS : single stranded

TOPOLOGY : linear

MOLECULE TYPE : DNA

ORIGINAL SOURCE : chemical synthesis

SEQUENCE DESCRIPTION : SEQ ID NO : 16 :

GGAATTCGAT ATCAAGCTTA TCGAT

INFORMATION FOR SEQ ID NO : 17

LENGTH : 28

TYPE : nucleic acid

STRANDEDNESS : single stranded

TOPOLOGY : linear

MOLECULE TYPE : DNA

ORIGINAL SOURCE : chemical synthesis

SEQUENCE DESCRIPTION : SEQ ID NO : 17 :

TCACCCGCCC TGGCCCTCTA GCTTCTCA

INFORMATION FOR SEQ ID NO : 18

LENGTH : 28

TYPE : nucleic acid

STRANDEDNESS : single stranded

TOPOLOGY : linear

MOLECULE TYPE : DNA

ORIGINAL SOURCE : chemical synthesis

SEQUENCE DESCRIPTION : SEQ ID NO : 18 :

GGACGCGTGG ATCCACTAGT TCTAGAGC

INFORMATION FOR SEQ ID NO : 19

LENGTH : 55

TYPE : nucleic acid
STRANDEDNESS : single stranded
TOPOLOGY : linear
MOLECULE TYPE : DNA
ORIGINAL SOURCE : chemical synthesis
SEQUENCE DESCRIPTION : SEQ ID NO : 19 :
TCATTTATCA TCATCATCTT TATAATCCCC GCCCTGGCCC TCTAGCTTCT CAGTG

INFORMATION FOR SEQ ID NO : 20
LENGTH : 37
TYPE : nucleic acid
STRANDEDNESS : single stranded
TOPOLOGY : linear
MOLECULE TYPE : DNA
ORIGINAL SOURCE : chemical synthesis
SEQUENCE DESCRIPTION : SEQ ID NO : 20 :
AACCATCCCC GAGGGTGTCT GCTGGAAGCC AGGCTCA

INFORMATION FOR SEQ ID NO : 21
LENGTH : 33
TYPE : nucleic acid
STRANDEDNESS : single stranded
TOPOLOGY : linear
MOLECULE TYPE : DNA
ORIGINAL SOURCE : chemical synthesis
SEQUENCE DESCRIPTION : SEQ ID NO : 21 :
CCTCTAGAGT CGCGGCCGTC GCACTCATTT ACC

INFORMATION FOR SEQ ID NO : 22
LENGTH : 29
TYPE : nucleic acid
STRANDEDNESS : single stranded

TOPOLOGY : linear
MOLECULE TYPE : DNA
ORIGINAL SOURCE : chemical synthesis
SEQUENCE DESCRIPTION : SEQ ID NO : 22 :
AAGGATCCCC GCCCTGGCCC TCTAGCTTC

INFORMATION FOR SEQ ID NO : 23
LENGTH : 36
TYPE : nucleic acid
STRANDEDNESS : single stranded
TOPOLOGY : linear
MOLECULE TYPE : DNA
ORIGINAL SOURCE : chemical synthesis
SEQUENCE DESCRIPTION : SEQ ID NO : 23 :
CCTCTAGACG CGTAGAGCGG CCGCCACCGC GGTGGA

INFORMATION FOR SEQ ID NO : 24
LENGTH : 25
TYPE : nucleic acid
STRANDEDNESS : single stranded
TOPOLOGY : linear
MOLECULE TYPE : DNA
ORIGINAL SOURCE : chemical synthesis
SEQUENCE DESCRIPTION : SEQ ID NO : 24 :
TCACACCTCA GTTGCTATGA CGCAC

INFORMATION FOR SEQ ID NO : 25
LENGTH : 28
TYPE : nucleic acid
STRANDEDNESS : single stranded
TOPOLOGY : linear
MOLECULE TYPE : DNA

ORIGINAL SOURCE : chemical synthesis
SEQUENCE DESCRIPTION : SEQ ID NO : 25 :
GGACGCGTGG ATCCACTAGT TCTAGAGC

INFORMATION FOR SEQ ID NO : 26
LENGTH : 51
TYPE : nucleic acid
STRANDEDNESS : single stranded
TOPOLOGY : linear
MOLECULE TYPE : DNA
ORIGINAL SOURCE : chemical synthesis
SEQUENCE DESCRIPTION : SEQ ID NO : 26 :
TCATTTATCA TCATCATCTT TATAATCCAC CTCAGTTGCT ATGACGCACT C

INFORMATION FOR SEQ ID NO : 27
LENGTH : 25
TYPE : nucleic acid
STRANDEDNESS : single stranded
TOPOLOGY : linear
MOLECULE TYPE : DNA
ORIGINAL SOURCE : chemical synthesis
SEQUENCE DESCRIPTION : SEQ ID NO : 27 :
CGCCGCAGCG ATGCGTTCCC CACGG

INFORMATION FOR SEQ ID NO : 28
LENGTH : 25
TYPE : nucleic acid
STRANDEDNESS : single stranded
TOPOLOGY : linear
MOLECULE TYPE : DNA
ORIGINAL SOURCE : chemical synthesis
SEQUENCE DESCRIPTION : SEQ ID NO : 28 :

GGAATTCGAT ATCAAGCTTA TCGAT

INFORMATION FOR SEQ ID NO : 29
LENGTH : 27
TYPE : nucleic acid
STRANDEDNESS : single stranded
TOPOLOGY : linear
MOLECULE TYPE : DNA
ORIGINAL SOURCE : chemical synthesis
SEQUENCE DESCRIPTION : SEQ ID NO : 29 :
TCAATCTGTT CTGTTGTTCA GAGGCCG

INFORMATION FOR SEQ ID NO : 30
LENGTH : 28
TYPE : nucleic acid
STRANDEDNESS : single stranded
TOPOLOGY : linear
MOLECULE TYPE : DNA
ORIGINAL SOURCE : chemical synthesis
SEQUENCE DESCRIPTION : SEQ ID NO : 30 :
GGACGCGTGG ATCCACTAGT TCTAGAGC

INFORMATION FOR SEQ ID NO : 31
LENGTH : 51
TYPE : nucleic acid
STRANDEDNESS : single stranded
TOPOLOGY : linear
MOLECULE TYPE : DNA
ORIGINAL SOURCE : chemical synthesis
SEQUENCE DESCRIPTION : SEQ ID NO : 31 :
TCATTTATCA TCATCATCTT TATAATCATC TGTTCTGTTG TTCAGAGGCC G

INFORMATION FOR SEQ ID NO : 32

LENGTH : 31

TYPE : nucleic acid

STRANDEDNESS : single stranded

TOPOLOGY : linear

MOLECULE TYPE : DNA

ORIGINAL SOURCE : chemical synthesis

SEQUENCE DESCRIPTION : SEQ ID NO : 32 :

AAGGATCCGT TCTGTTGTTC AGAGGCCGCC T

INFORMATION FOR SEQ ID NO : 33

LENGTH : 36

TYPE : nucleic acid

STRANDEDNESS : single stranded

TOPOLOGY : linear

MOLECULE TYPE : DNA

ORIGINAL SOURCE : chemical synthesis

SEQUENCE DESCRIPTION : SEQ ID NO : 33 :

CCTCTAGACG CGTAGAGCGG CCGCCACCGC GGTGGA

INFORMATION FOR SEQ ID NO : 34

LENGTH : 28

TYPE : nucleic acid

STRANDEDNESS : single stranded

TOPOLOGY : linear

MOLECULE TYPE : DNA

ORIGINAL SOURCE : chemical synthesis

SEQUENCE DESCRIPTION : SEQ ID NO : 34 :

CTATACGATG TACTCCATTC GGTTTAAG

INFORMATION FOR SEQ ID NO : 35

LENGTH : 31

TYPE : nucleic acid
STRANDEDNESS : single stranded
TOPOLOGY : linear
MOLECULE TYPE : DNA
ORIGINAL SOURCE : chemical synthesis
SEQUENCE DESCRIPTION : SEQ ID NO : 35 :
GGACGCGTCT AGAGTCGACC TGCAGGCATG C

INFORMATION FOR SEQ ID NO : 36
LENGTH : 52
TYPE : nucleic acid
STRANDEDNESS : single stranded
TOPOLOGY : linear
MOLECULE TYPE : DNA
ORIGINAL SOURCE : chemical synthesis
SEQUENCE DESCRIPTION : SEQ ID NO : 36 :
CTATTTATCA TCATCATCTT TATAATCTAC GATGTACTCC ATTCGGTTTA AG

Claims

1. A polypeptide comprising amino acid sequence of the SEQ ID NO. 1 of the sequencing list encoded in a gene of the human origin.

2. A polypeptide comprising at least amino acid sequence of the sequence identification SEQ ID NO. 2 or 5 of the sequencing list.

3. The polypeptide according to claim 2 comprising amino acid sequence of SEQ ID NO. 3 of the sequncing list.

4. The polypeptide according to claim 2 comprising amino acid sequence of SEQ ID NO. 4 of the sequncing list.

5. The polypeptide according to claim 2 comprising amino acid sequence of SEQ ID NO. 6 of the sequncing list.

6. The polypeptide according to claim 2 comprising amino acid sequence of SEQ ID NO. 7 of the sequncing list.

7. The polypetide according to any one of claims 1 to 6 having differentiation suppressive action against undifferentiated cells.

8. The polypeptide according to claim 7 wherein the undifferentiated cells are the undifferentiated cells except for those of the brain and nervous system or muscular system cells.

9. The polypetide according to claim 7 wherein the undifferentiated cells are undifferentiated blood cells.

10. A pharmaceutical composition comprising containing the polypeptide of any one of claims 1 to 6.

11. The pharmaceutical composition according to claim 10 wherein use thereof is hematopoietic activator.

12. A cell culture medium comprising containing the polypeptide of any one of claims 1 to 6.

13. The cell culture medium according to claim 12 wherein the cell is the undifferentiated blood cell.

14. A DNA coding a polypeptide comprising at least having amino acid sequence of SEQ ID NO. 2 or 5 of the sequencing list.

15. The DNA according to claim 14 comprising having DNA sequence 242-841 of SEQ ID NO. 8 or DNA sequence 502-1095 of SEQ ID NO. 9 of the sequencing list.

16. The DNA according to claim 14 comprising coding the polypeptide having amino acid sequence of SEQ ID NO. 3 of the sequencing list.

17. The DNA according to claim 16 comprising having DNA sequence 242-1801 of SEQ ID NO. 8 of the sequencing list.

18. The DNA according to claim 14 comprising coding the polypeptide having amino acid sequence of SEQ ID NO. 4 of the sequencing list.

19. The DNA according to claim 18 comprising having DNA sequence 242-2347 of SEQ ID NO. 8 of the sequencing list.

20. The DNA according to claim 14 comprising coding the polypeptide having amino acid sequence of SEQ ID NO. 6 of the sequencing list.

21. The DNA according to claim 20 comprising having DNA sequence 502-3609 of SEQ ID NO. 9 of the sequencing list.

22. The DNA according to claim 14 comprising coding the polypeptide having amino acid sequence of SEQ ID NO. 7 of the sequencing list.

23. The DNA according to claim 22 comprising having DNA sequence 502-4062 of SEQ ID NO. 9 of the sequencing list.

24. A recombinant DNA comprising formed by ligating a DNA selected from the group of DNAs of claims 14 to 23 and a vector DNA which can express in the host cell.

25. A cell comprising transformed by the recombinant DNA according to claim 24.

26. A process for production of the polypeptide of any one of claims 1 to 6 comprising culturing cells of claim 25 and isolating the compound produced in the cultured mass.

27. An antibody specifically recognizing the polypeptide having the amino acid sequence of SEQ ID NO. 4 of the sequencing list.

28. An antibody specifically recognizing the polypeptide having the amino acid sequence of SEQ ID NO. 7 of the sequencing list.

FIG. 1

```
Cosensus          :**C***YY***C***CRPRDD*FGH**C***G***C**GW*G**C
hDelta-1.DSL      :FVCDEHYYGEGCSVFCRPRDDAFGHFTCGERGEKVCNPGWKGPYC
dDelta.DSL        :VTCDLNYYGSGCAKFCRPRDDSFGHSTCSETGEIICLTGWQGDYC
xDelta.DSL        :FVCDEYYYGEGCSDYCRPRDDAFGHFSCGERGEKLCNPGWKGLYC
cDelta-1.DSL      :FVCDEHYYGEGCSVFCRPRDDRFGHFTCGERGEKVCNPGWKGQYC
mDelta-1.DSL      :FVCDEHYYGEGCSVFCRPRDDAFGHFTCGDRGEKMCDPGWKGQYC
hSerrate-1.DSL    :VTCDDYYYGFGCNKFCRPRDDFFGHYACDQNGNKTCMEGWMGPEC
dSerrate.DSL      :VQCAVTYYNTTCTTFCRPRDDQFGHYACGSEGQKLCLNGWQGVNC
rJagged.DSL       :VTCDDHYYGFGCNKFCRPRDDFFGHYACDQNGNKTCMEGWMGPEC
                     *    **    *    ****** ***  *    *    *  ** *  *
```

FIG 2

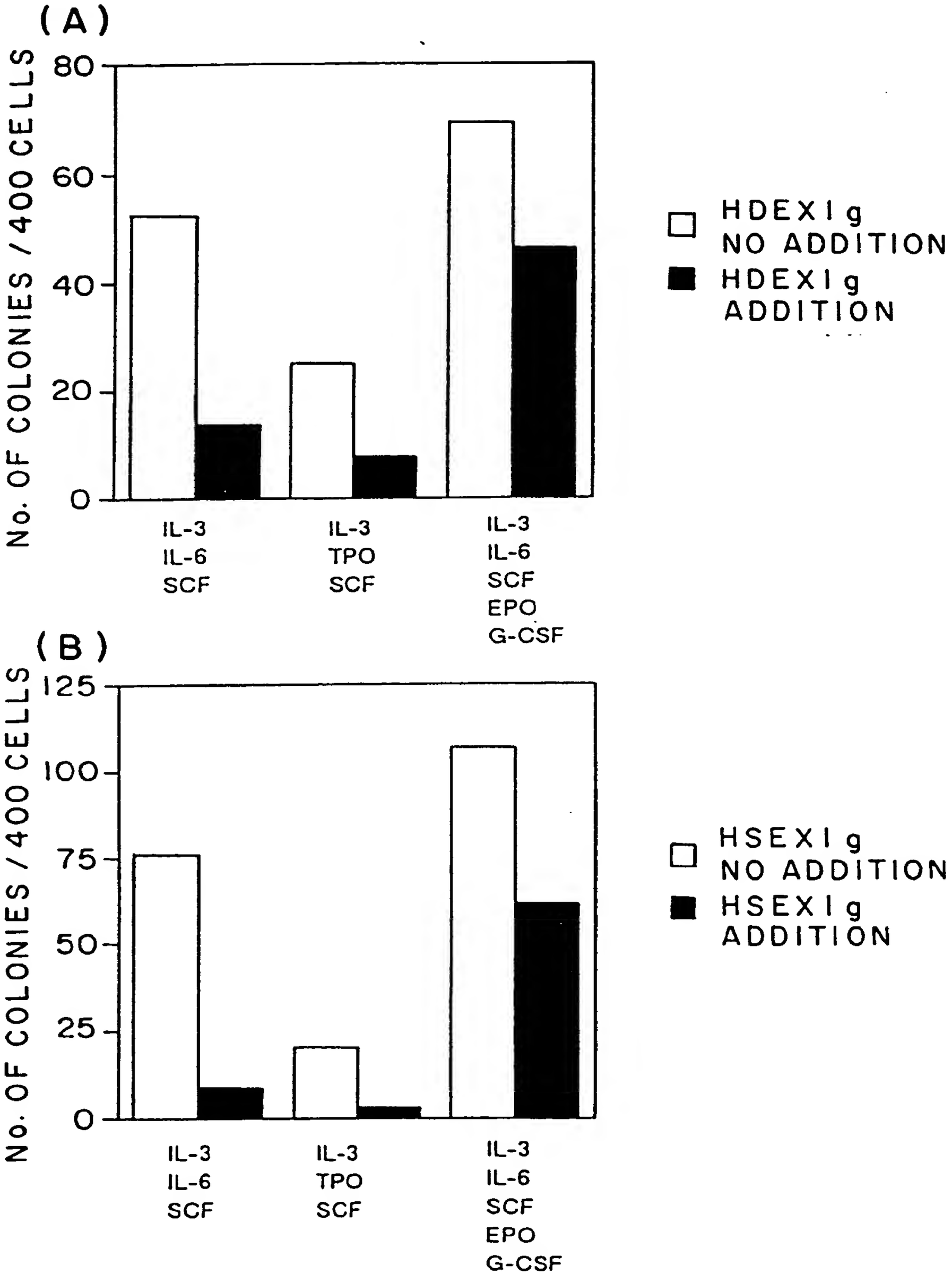


FIG. 3

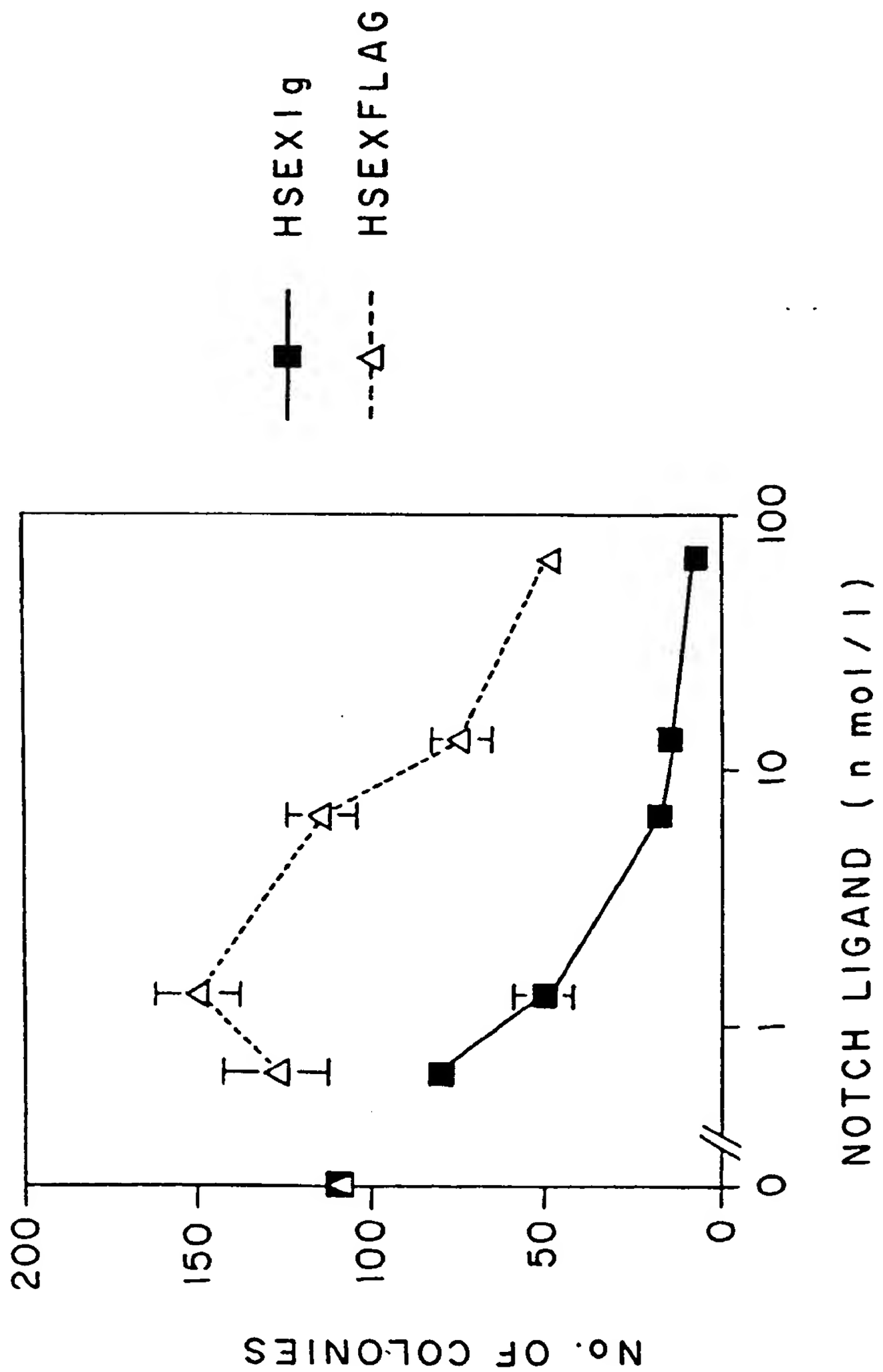


FIG. 4

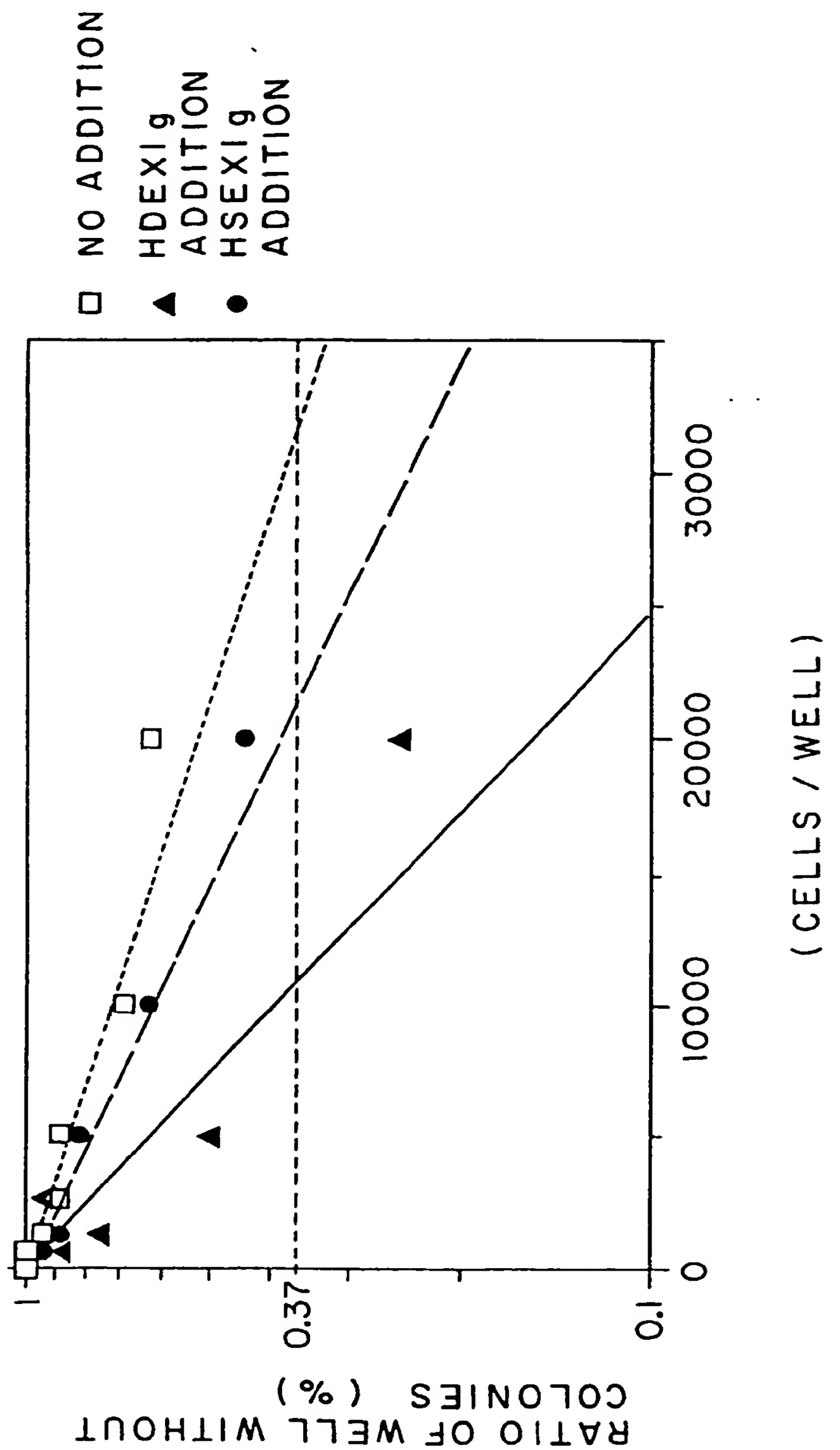
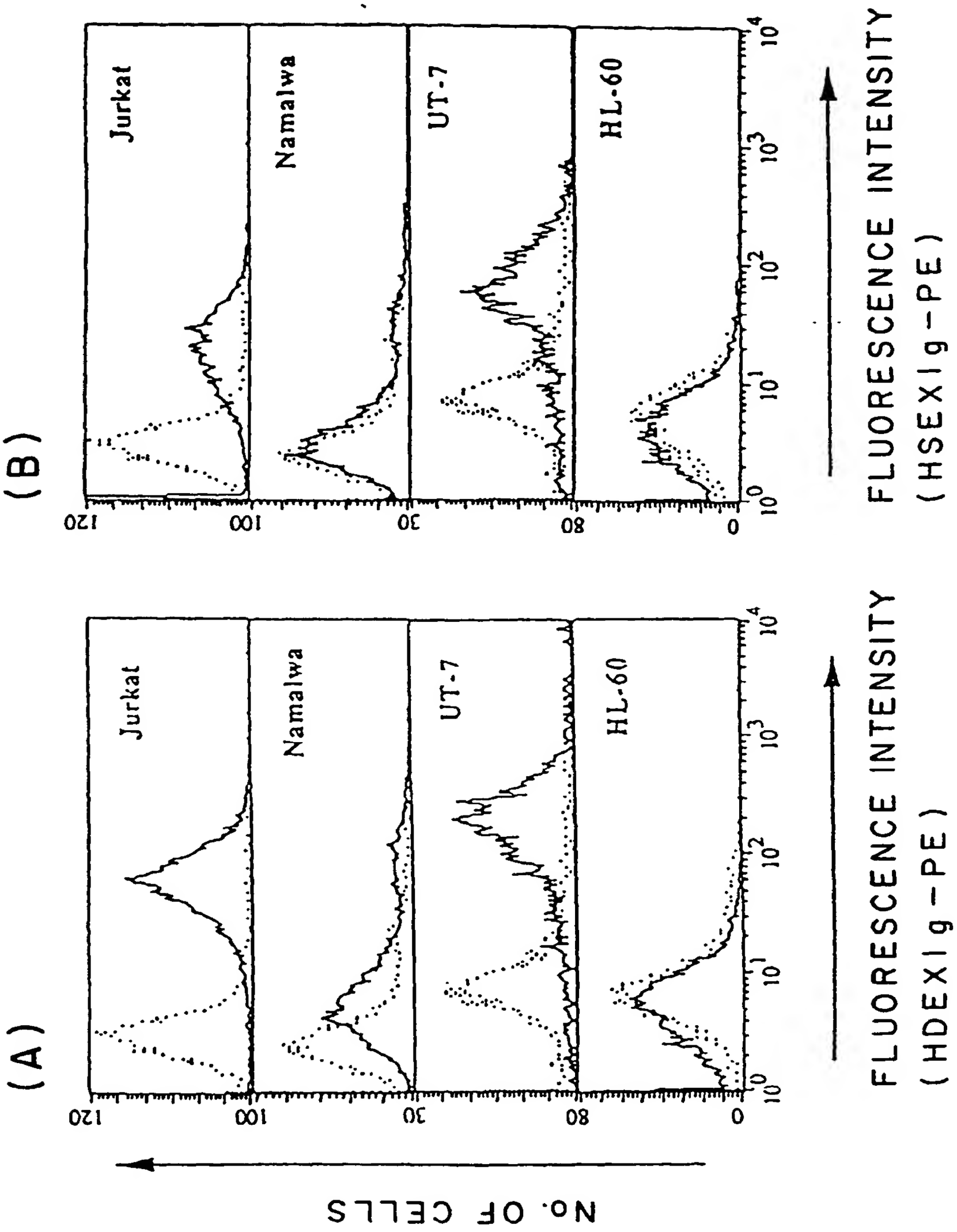


FIG. 5



INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP96/03356

A. CLASSIFICATION OF SUBJECT MATTER
Int. Cl[6] C12N15/19, C12N15/63, C07K14/52, C12N5/00, C12N5/18, C12P21/02, C07K16/24, C12P21/08, A61K38/19 // (C12P21/02, C12R1:91)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Int. Cl[6] C12N15/19, C12N15/63, C07K14/52, C12N5/00, C12N5/18, C12P21/02, C07K16/24, C12P21/08, A61K38/19

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
GENETYX-MAC/CD, WPI, WPI/L, BIOSIS PREVIEWS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GENES Dev. 4 (1990) R.J. Fleming et al. "The gene Serrate encodes a putative EGF-like transmembrane protein essential for proper ectodermal development in Drosophila melanogaster" p. 2188-2201	1 - 28
A	Development 111 (1991) U. Thomas et al. "The Drosophila gene Serrate encodes an EGF-like transmembrane protein with a complex expression pattern in embryos and wing discs" p. 749-761	1 - 28
A	EMBO J. 6(11)(1987) H. Vassin et al. "The neurogenic gene Delta of Drosophila melanogaster is expressed in neurogenic territories and encodes a putative transmembrane protein with EGF-like repeats" p. 3431-3440	1 - 28
A	Development 121(8)(08.1995) B. Bettenhausen et al. "Transient and restricted expression during mouse embryogenesis of D111, a murine gene	1 - 28

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
February 6, 1997 (06. 02. 97)	February 18, 1997 (18. 02. 97)
Name and mailing address of the ISA/ Japanese Patent Office Facsimile No.	Authorized officer Telephone No.

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP96/03356

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	closely related to Drosophila Delta" p. 2407-2418	
A	Nature 375(06.1995) D. Henrique et al. "Expression of a Delta homologue in prospective neurons in the chick" p. 787-790	1 - 28
A	Nature 375(06.1995) A. Chitnis et al. "Primary neurogenesis in Xenopus embryos regulated by a homologue of the drosophila neurogenic gene Delta" p. 761-766	1 - 28
A	Cell 80(6)(24.03.1995) C.E. Lindsell et al. "Jagged:A Mammalian Ligand That Activates Notch1" p. 909-917	1 - 28

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FIG. 1

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Cosensus          :**C***YY***C***CRPRDD*FGH**C***G***C**GW*G**C
hDelta-1.DSL      :FVCDEHYYGEGCSVFCRPRDDAFGHFTCGERGEKVCNPGWKGPYC
dDelta.DSL        :VTCDLNYYGSGCAKFCRPRDDSFGHSTCSETGEIICLTGWQGDYC
xDelta.DSL        :FVCDEYYYGEGCSDYCRPRDDAFGHFSCGERGEKLCNPGWKGLYC
cDelta-1.DSL      :FVCDEHYYGEGCSVFCRPRDDRFGHFTCGERGEKVCNPGWKGQYC
mDelta-1.DSL      :FVCDEHYYGEGCSVFCRPRDDAFGHFTCGDRGEKMCDPGWKGQYC
hSerrate-1.DSL    :VTCDDYYYGFGCNKFCRPRDDFFGHYACDQNGNKTCMEGWMGPEC
dSerrate.DSL      :VQCAVTYYNTTCTTFCRPRDDQFGHYACGSEGQKLCLNGWQGVNC
rJagged.DSL       :VTCDDHYYGFGCNKFCRPRDDFFGHYACDQNGNKTCMEGWMGPEC
                      *    **    *    ****** ***  *    *    *  ** *  *
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FIG 2

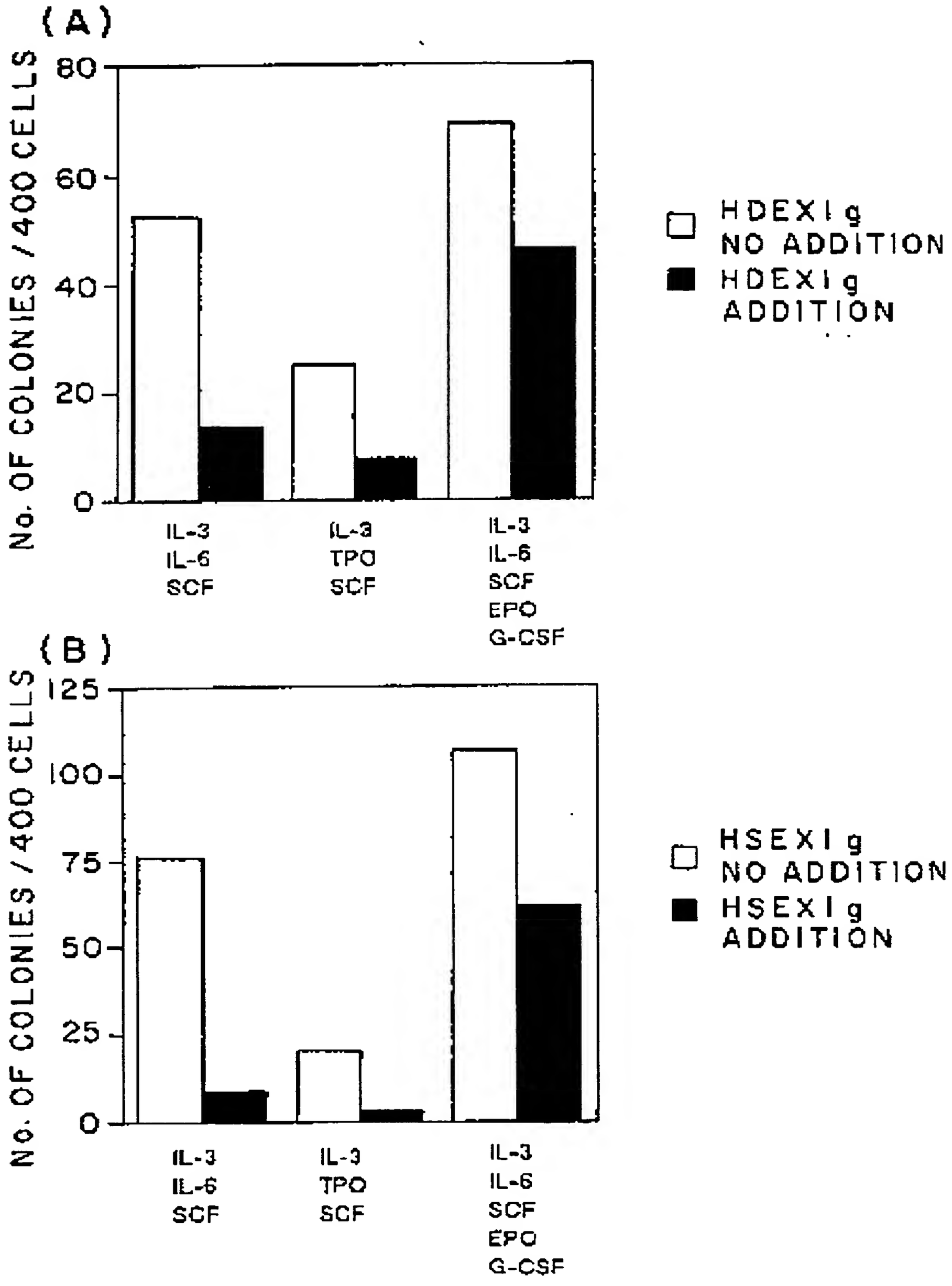

FIG. 3

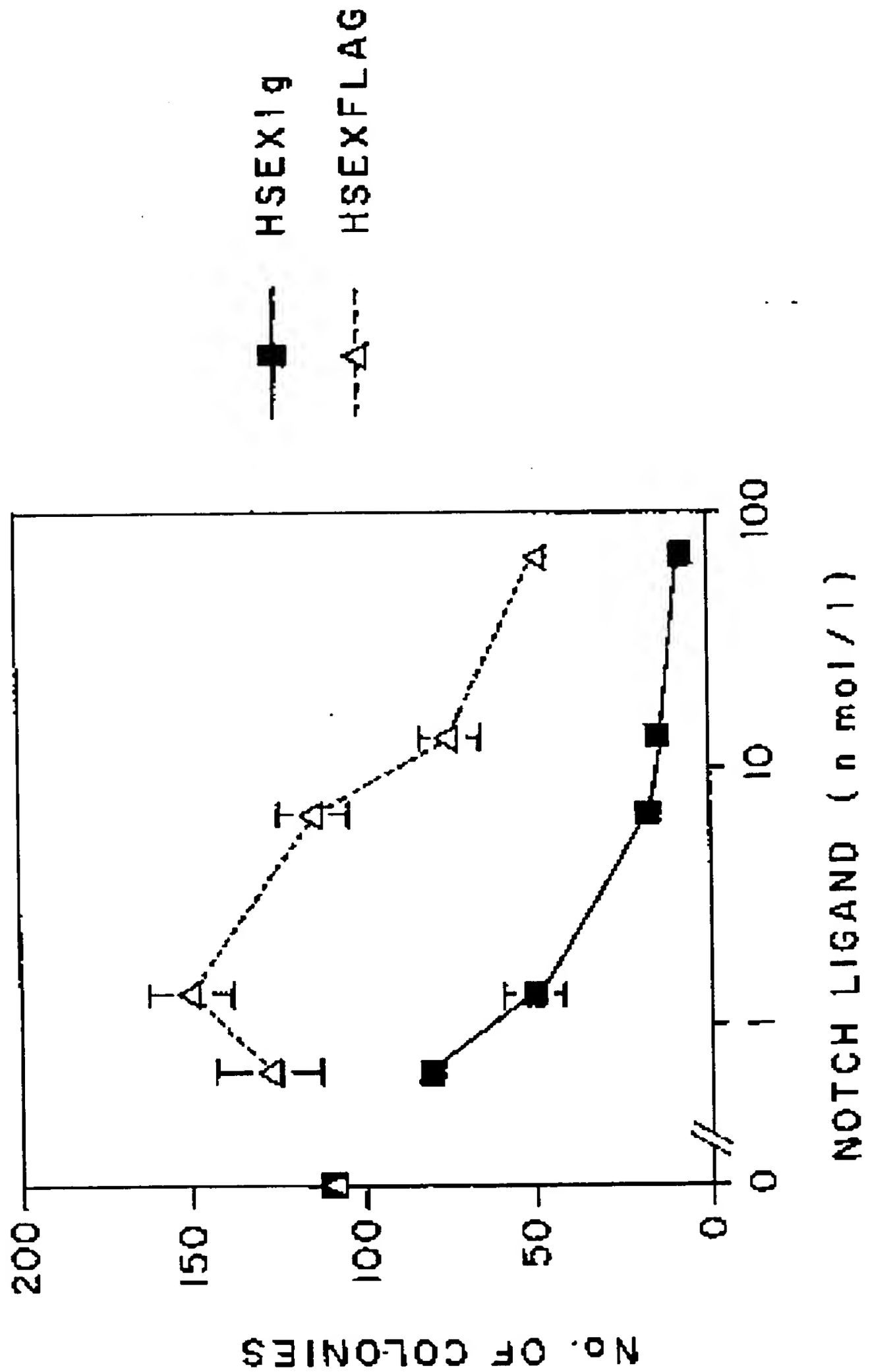

FIG. 4

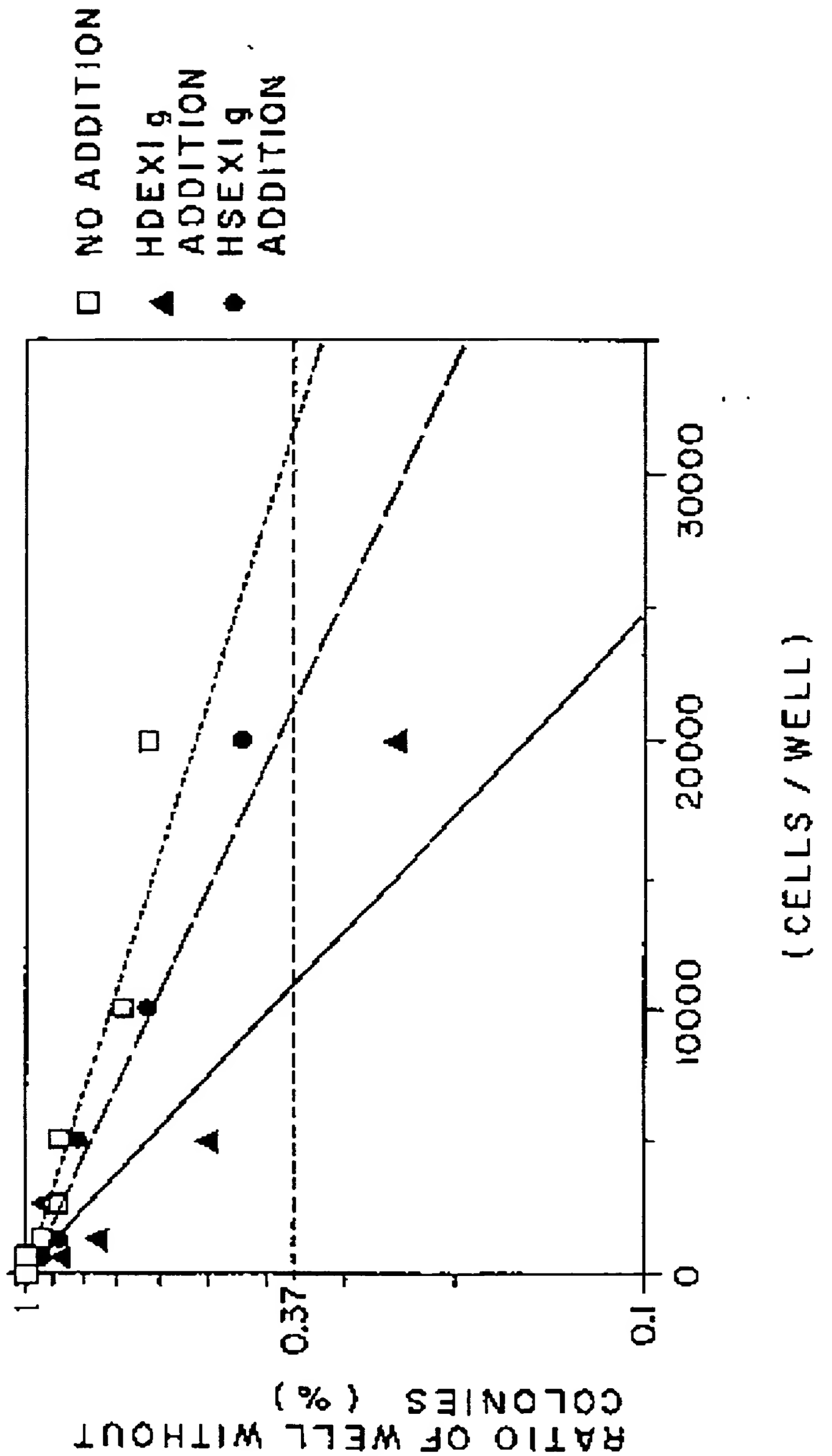

FIG. 5